CORNUS MAS L. CULTIVAR SELECTION BASED ON HARDINESS AND PROPAGATION

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Cornus mas L. cultivar selection based on hardiness and propagation

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ABSTRACT

Cornus mas (Cornelian cherry) is a deciduous shrub/small tree native to southeastern Europe and western Asia. It is unique among the Cornaceae (dogwood) family in that the fruit is used for human consumption and is highly nutritious and contains high amounts of antioxidants and anthocyanins. While this plant has many desirable fruiting and ornamental characteristics, it has seen limited use in North America. Several of these desirable characteristics are low disease and pest incidence, fruit qualities, and early yet long-lasting flowers. With limited use in North America, hardiness speculations are based on only a few individual plants. In this document, artificial freeze tests were conducted to better understand hardiness of the species and how vulnerable plants are when coming out of dormancy in the spring. Propagation methods (micropropagation, *in vitro* and *ex vitro* rooting of plantlets, and grafting) were also evaluated to determine the optimal method of growing cultivars.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BA 6-Benzylaminopurine

CRD..... Completely Randomized Design

DD5 ..... Degree days above 5 °C

EC..... Electrical conductivity

GLM ..... General Linear Model

IBA..... Indole-3-butyric acid

LP ..... Plant tissue culture medium described in Long et al. (1995)

MS ..... Plant tissue culture medium described in Murashige and Skoog (1962)

NAA ..... Naphthaleneacetic acid

PGR ..... Plant growth regulator (ie. cytokinins, auxins, etc.)

PPM..... Parts per million

RCBD ..... Randomized Complete Block Design

WPM ..... Woody Plant Media; a plant tissue culture medium described in Lloyd and McCown (1980)

CHAPTER 1. INTRODUCTION

Cornelian cherry dogwood (*Cornus mas* L.) is a deciduous shrub or small tree in the family Cornaceae (the dogwood family). Height usually ranges from 6 to 7.5 m with a spread of 4.5 to 6 m (Dirr, 2009). Native to eastern Europe and western Asia, this species is closely related to many ornamental dogwood species planted in landscapes across the United States (including *C. florida*, *C. kousa*, *C. alternifolia*, *C. racemosa*, etc.). Members of this taxonomic family are primarily used for aesthetics in ornamental landscapes. Flowers of *C. mas* are less showy than other members of the family such as *C. kousa* and *C. florida*, however the small yellow flowers cover the tree (Figure 1-1A) and are some of the earliest woody tree species flowers to emerge in the spring and may last up to three weeks (Dirr, 2009).

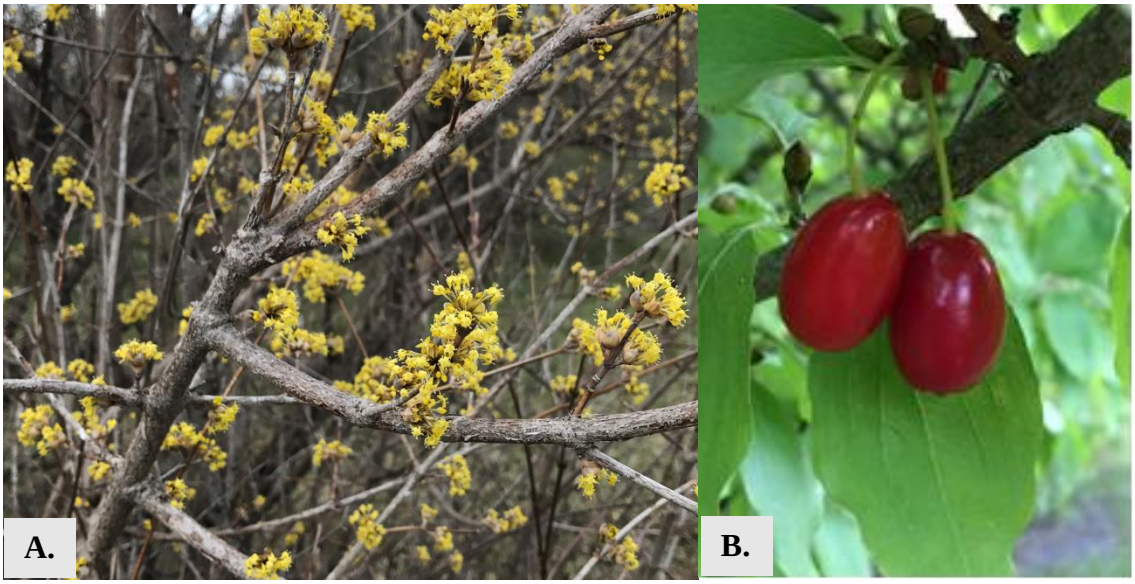

Figure 1-1. *Cornus mas* is one of the first plants to flower after winter. The flowers (shown on the right, A.) are borne in umbels prior to emergence (photo by Gregory Morgenson, NDSU). The fruit at right (B.) is a drupe containing an elongate seed (photo by Meredith Swanson, NDSU).

Unlike other members of its family, *C. mas* is prized (near its center of origin in eastern Europe) for the culinary uses of its fruit (shown in Figure 1-1B). This species is underutilized in the United States and has been for the most part ignored in modern North American breeding

programs. Some cultivars have been developed; however, the species is not commonly used in the United States. It is reported that the ancient Romans used this species for food consumption and medicinal purposes (Weaver, 1976). The Romans are also the group of people who are speculated to have spread the species for cultivation. Some heirloom cultivars (specifically those from Poland such as 'Kotula', 'Juliusz', 'Florianka', etc.) have been in use since the 1800s (N. Piórecki, personal communication, May 22, 2017). Klimenko (2004) reports that there has recently been a resurgence of breeding for the species in "Ukraine, Bulgaria, Slovakia, Austria, Yugoslavia, France, Germany, Poland and Turkey". The species can be found in the Mediterranean, the former Soviet Bloc, and middle eastern countries such as Iran and Turkey. Countries in these areas have extensive native stands, especially in Turkey where wild, open-pollinated plants make up 97% of the *C. mas* population and contribute to a wide array of genetic diversity (Ercýslý 2004).

C. mas fruit can be described in taste somewhere between a sour cherry and cranberry and varies from yellow to dark red in color (Klymenko, 2013). West et al. (2012) used degrees Brix (°Bx) to describe average sugar content of the cultivars used in their study, which can be compared to the °Bx of relatable fruits (Table 1-1.). Cultivars are generally lumped together in categories based on fruit color: yellow, red, and dark red. The yellow cultivar 'Flava' has even been described as tasting like pineapple (Klimenko, 2004). It is speculated that the yellow-fruited selections descend from a single wild yellow-fruited mutant. Klimenko (2004) cites that even as early as 1843 "varieties with yellow fruits were very rare" in the wild. It is thought that wild genotypes no longer exist in their natural habitat. Size of fruits may be around 3-5 cm in length and shape vary from rounded, to oval, to even pear shaped. The main qualities aimed for in breeding are fruit taste, size, quality, and plant hardiness. *Cornus mas* is, as Dirr (2009)

describes, “a very pest-free plant”. Klimenko (2004) likewise says that the species is “rarely attacked by pests and diseases”. With very few diseases and pests, breeders focus mainly on fruit characteristics. Fruits can be made into jams, jellies, wines, and sauces and have been utilized in western Asia for medicinal properties (Klimenko, 2004). Nutritional qualities (high antioxidants, anthocyanins, etc.) give this species added benefits (Ercýslý, 2004). It is for these reasons that *C. mas* has high potential for ediscaping (edible landscaping) in North America. Low pest and disease incidence give the species added value (Klimenko, 2004), with powdery mildew and anthracnose being the only diseases of concern. This is unlike edible and ornamental plants in the Rosaceae family (apples, cherries, plums, roses, potentilla, etc.) which are constantly plagued with diseases and pests such as fireblight, viruses, and various fungal and bacterial issues unless otherwise bred for resistance. Adaptable to many pH conditions, *C. mas* transplants well and can be a good choice for shrub borders and hedges. The fruit is eaten by many animals and birds, which can be of value in wildlife plantings. If the fruit persists on the plant, it can add ornamental attraction, thus its potential for ediscaping.

Table 1-1. Total soluble solids of fruit comparable to *Cornus mas*

Fruit	Brix value (°Brix)
Sweet cherry (*Prunus avium* L.)	20
Cornelian cherry (*C. mas*)*	**15**
Sour cherry (*Prunus cerasus* L.)	14
Pineapple (*Ananas comosus* L.)	12.8
Cranberry (*Vaccinium macrocarpon* Aiton)	7.5
Japanese cornel (*C. officinalis* Torr. ex Dur)*	6.55
Lemon (*Citrus limon* [L.] Osbeck)	4.5

* Cornelian cherry value obtained from Dokoupil and Řezníček, 2013; Japanese cornel value obtained from West et al., 2012. All other items in table are found in the Electronic Code of Federal Regulations, Title 21, Volume 2, Subpart B, Revised April 2, 2012.

Hardiness

With all the attributes of *C. mas* it is unknown why it is an uncommon species in the U.S. and fruit breeding programs or why it has not been evaluated for its tolerance to northern climates on this continent. There is a large potential for cultivars to become popular as small fruits for production as well as ediscaping as previously stated. Weaver (1976) reports that the species is available in multiple nurseries in the U.S., however the author was "unable to find sources for any of the cultivars". While Weaver published this statement over 40 years ago, only a few select cultivars can be found in production in the U.S. with the majority of germplasm and selections from Europe being present only in private collections and arboretums. Uncommon cultivars are far more publicly accessible in Europe than in the United States. With the relatively few studies done on the species in the U.S., it is highly important for hardiness testing to be conducted. Many sources and publications, and even nurseries, conflict with each other as to whether the species falls into USDA (U. S. Department of Agriculture) cold hardiness zones 4 or 5. The USDA cold hardiness zone map divides the U.S. into thirteen zones that are based on average annual minimum winter temperature. Zone 4 annual minimum temperature reaches -30 to -20°F (-34.4 to -28.9°C) while zone 5 reaches -20 to -10°F (-28.9 to -23.3°C). These zones are a standard that aid plant producers and landscapers (as well as home gardeners) in choosing appropriate plants to grow in their respective regions without risk of complete plant death during winter. Table 1-2. shows *Cornus* species relative to the zones they belong to. It has been observed that a cultivar ('Variegata') at the University of Wisconsin-Madison Arboretum (Madison, WI, USA; 43.0416, Long: -89.4311; USDA hardiness zone 5a \) suffers winter damage every year and overall is a very poor plant selection for that region (D. Stevens, personal communication). This is contrary to an individual accession, TS79239, of *C. mas* established

since 1979 at the NDSU Dale E. Herman Research Arboretum (Absaraka, ND, USA; Lat: 46.9859, Long: -97.3549; USDA hardiness zone 4a) that suffered minimal cold damage and has survived many harsh winters down to -35 °F (-37.2 °C). A second individual at this same location (TS7987), while having survived since its planting, is less hardy than TS79239 and shows stem dieback after coming out of dormancy in spring (T. West, personal communication). A further unknown related to plant hardiness is flower bud hardiness of accessions. TS79239, which produces flower buds yearly, has only produced fruit in 2017 and not prior. This may be attributed to partial self-infertility (Reich, 2007), as flowers only formed fruit after being exposed to the pollen of a different accession or cultivar. If bud hardiness is an issue with flower buds as well, it should be confirmed by testing. The second individual (TS7987) at the same location flowers, has never produced fruit, and exhibits dieback after winter. Ornamental plants diminish their landscape potential if flowers don't bloom in the spring and then fail to set fruit in summer. The hardiness ratings for differing cultivars must be established in order for recommendations to be made to landscapers, homeowners, and nursery growers for proper plant placement in specific climate types. Cappiello and Shadow (2005) give approximate hardiness for only five of the twenty-six cultivars mentioned in their book and this is largely based on observations of individual plants dying after winters of documented temperatures. Likewise, Dirr (2009) only lists approximate hardiness for four of the twenty-one cultivars mentioned in his publication (with some of this information being cited from Cappiello and Shadow, 2005). These observations also only constitute a very small portion of *C. mas* cultivars. In 2015, approximately 48 selections had passed through the NDSU Woody Plant Improvement Program in the form of grafting material, *in vitro* cultures, and material for freeze testing. Of these, few have hardiness information attributed to them from other sources. Doing controlled freeze testing

on many cultivars and as well as across tissue types (i.e. stems, flower buds, and vegetative buds) has potential to give approximate hardiness scores to cultivars that have previously not been labeled for USDA cold hardiness zones. Estimating, and then confirming through field testing, the hardiness of cultivars would give the U.S. more options in utilizing this species.

Table 1-2. *Cornus* ssp. cold hardiness

USDA hardiness zone	Average minimum temperature (°C)	Average minimum temperature (°F)	Related *Cornus* species
2	-45.6 to -40	-50 to -40	*C. canadensis*
			C. sericea
3	-40 to -34.4	-40 to -30	*C. alternifolia*
			C. alba
			C. racemosa
4	-34.4 to -28.9	-30 to -20	*C. amomum*
			C. mas
			C. sanguinea
5	-28.9 to -23.3	-20 to -10	*C. kousa*
			C. florida
			C. macrophylla
			C. officinalis
6	-23.3 to -17.8	-10 to 0	
7	-17.8 to -12.2	0 to 10	*C. nuttallii*
8	-12.2 to -6.7	10 to 20	*C. capitata*
9	-6.7 to -1.1	20 to 30	*C. chinensis*

USDA cold hardiness zone information for species from Dirr (2009).

Controlled freeze testing as mentioned above has been previously used in other species to determine plant potential in different USDA cold hardiness zones. Aitken and Adams (1997) used controlled freezing to determine differences between two coastal populations of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco]. Shoot tips were sampled from four sites and, using artificial freezing at controlled temperature intervals and visually examining samples to score tissue death, the individual cold hardiness was determined. Visually ranking of damage in this manner is relative to the individual researcher evaluating samples and may not be accurate. Their objective was to focus on spring hardiness and susceptibility to latent frost. Because of this,

tissue samples were taken in March and April of 1993 as well as April of 1994. They found that genotypes of families of Douglas-fir greatly influence spring cold hardiness. The authors also concluded that the artificial freezing process they used in their research was consistent and efficient, making it a useful tool in both breeding for and selecting spring hardiness in the species. The main disadvantageous factor in their study was that the only way to determine actual damage to the sampled tissue was visual observations. This could skew results because of human error. While two people conducted the visual rankings of damage, the samples in the same replication were evaluated by the same person. This could still lead to skewed data between replications. For an experiment resulting in less error, a more mechanized means of measuring damage should be used. Aitken and Adams (1996) have also replicated this experiment in regard to fall and winter hardiness evaluation and found the procedures to be acceptable in those seasons as well for early frost tolerance.

Controlled freeze testing has also been implemented by Hamilton et al. (2016) to evaluate white spruce [*Picea glauca* (Moench) Voss]. The procedures in this experiment closely followed those of Aitken and Adams (1996, 1997) except that instead of visual ranking of damage, electrolyte leakage was measured as a more accurate way to determine cold damage. To determine electrolyte leakage, electrical conductivity (EC) was measured after samples were removed from artificial freeze conditions and stored at 4 °C for 24 h. Electrical conductivity was again measured after complete death of the tissue was achieved by exposing to 95 °C for 1 h and stored at 4 °C for 24 h. The ratio of measurements after freezing and after the heat kill can be used to determine percentage of electrolytes leaked into the solution and therefore the amount of tissue death caused by freezing. This mechanized method for determining tissue death is a

superior method to the visual ranking system as used by Aitken and Adams (1996, 1997) and leaves far less room for error or variation between different individuals recording data.

Cold hardiness models have also been used in evaluating grape canes and buds. Miller et al. (2006) used artificial freezing to evaluate hardiness in multiple grape cultivars. Rather than using EC or relying on visual rating alone, thermoelectric modules (TEMs) were used to identify at which temperatures tissue samples underwent low temperature exotherms (LTE). An LTE can be described as "a temperature below the killing temperature as detected by differential thermal analysis". This percentage could be correlated to the percentage of tissue death, respectively. Then, tissue samples were inspected at the LTE ratings indicated for frost damage. Another study on grape bud hardiness (Quamme, 1986) used thermocouples in measuring frost damage. However, the limitation of this study was that the cooling rates of tissue samples were 1.5, 5, 10, and 40 °C/h. Mills et al. (2006) state that "rapid cooling rates (for example, 10 °C/h) are not realistic in nature and not representative of natural freezing events". Mills et al. used a cooling rate of 4 °C/hr instead. It is likely that the 40 °C/h cooling treatment in Quamme's study are unrealistic and have limited applications. Even though there are limited applications, this research is still a valid example of controlled freezing's use in applied research.

Propagation

A novel species such as *Cornus mas* requires a viable clonal propagation method for cultivars to be produced and distributed to consumers. Methods for propagating *C. mas* includes grafting, vegetative stem cuttings, and plant tissue culture (micropropagation). In Turkey, the vast native stands are all the product of open pollinated seed (Ercýslý, 2004). This has resulted in a wide array of germplasm and genetic diversity (explained by diversity in fruit shape, color, size, flavor, nutritional value, etc.; Ercýslý, 2004). Ercýslý (2004) reported that 97% of the *C.*

mas individuals in Turkey are a result of these wild stands and natural gene pool. Only 3% of *C. mas* individuals in that country are commercially propagated through clonal grafting of cultivars. Seed propagation, while valuable for producing crosses, is mainly valuable for the production of rootstocks for grafting. Dirr (2009) gives directions for stratifying seeds, which is useful information for people wishing to propagate crosses. Seed does not produce genetically identical plants and therefore cannot be used directly for clonal production of cultivars that would be utilized in the nursery and landscaping industry. Also, for a species that is partially self-infertile (such as *C. mas*), it may be difficult to self-pollinate plants to breed pure lines (inbred seed lineages exhibiting genetic homogeneity; Reich, 2007). Layering, or the wounding and burying of stems still connected to a mother plant to form adventitious roots, only has applications for propagating mature individuals rather than the small plantlets from *in vitro* tissue culture conditions. This being said, layering has been reported by Ďurkovič (2008) to be an unguaranteed means of successful propagation. Therefore, layering may not be feasible for commercial use even for the propagation of mature individuals.

Grafting

Grafting refers to the practice joining two plants together via the vascular tissue (cambium): a seedling rootstock and the donor cultivar, or "scion" material. This is done to mediate limitations to other forms of propagation (Beyl and Trigiano, 2008). Grafting produces genetically uniform plants and is used rather than seed to produce clonal selections (cultivars and varieties). In some cases, species do not root well for cutting propagation. Grafting eliminates the need for root formation from the selection being propagated. In other cases, rootstocks resistant to certain diseases are utilized to provide beneficial immunity to said diseases for the selection being produced. There are many different types of grafts that are utilized in horticulture (cleft,

side, chip budding, etc). Grafting (cleft or side) utilizes one or more buds on a section of woody stem whereas budding uses only one bud attached to a small amount of bark of the donor plant. Preferable graft type/methods depend on the species being produced. Grafting and budding are widely used for tree fruit production as well as for cultivars in the ornamental horticulture industry (Beyl and Trigiano, 2008).

Dirr and Heuser (1987) reported that there are no mentions of grafting methods found in the literature for *C. mas*. Ercýslý (2004) reported that the 3% of Turkey's *C. mas* population is propagated by grafting cultivars but fails to cite where this information comes from or the methods used to graft. The NDSU Woody Plant Improvement Program utilized grafting to propagate cultivars acquired from various sources but no comparison of grafting types and methods were initially conducted. While not all grafts were successful, grafting propagation has resulted in a collection of cultivars bred from different parts of the species' native range. This led to the belief that further studies done on grafting of *C. mas* will provide a reliable form of propagation. Bijelić et al. (2016) found that a maximum success rate of around 76-84% could be obtained by budding. They concluded that "quality planting material" can be obtained from the five accessions (CPC16, APRANI, BACKA, R1, and PPC1) studied and therefore budding should be considered for mass production.

Tissue Culture

Tissue culture, which may also be referred to as *in vitro* micropropagation, is the proliferation of a plant or plant parts in laboratory conditions, a sterile environment, on synthetic aseptic medium. Plant tissue (typically axillary buds/shoots) is used for mass production of clonal plants (typically a cultivar or variety) in horticulture and "has become a standard tool for the nursery trade" (Beyl and Trigiano, 2008). Additional benefits of using tissue culture as a

method for propagation include elimination of disease and the production of vigorous plants. According to Trigiano and Gray (2011), most growth rooms are set up to provide ideal growing conditions and equipped with cool white florescent lights and set to 26 to 28 ℃. As with other methods and aspects of propagation, tissue culture conditions will vary depending on the species being produced and that species' growing preferences. The media that plantlets are grown on can vary in its macro and micronutrition, plant growth regulators (PGRs, synthetic hormones) and concentrations, sugars, organic components (such as vitamins), and any additional gelling agents for plant support. PGRs are one of the main components in a growing medium that initiate and control growth and development of shoots, roots, and callus (undifferentiated cells). Common PGRs that are used include auxins and cytokinins which play roles in cell elongation and division, respectively (Trigiano and Gray, 2011).

There have been experiments resulting in somatic embryo genesis of *C. mas* from seedlings and zygotic embryos (as cited in Ďurkovič, 2008). The term "somatic embryo genesis" refers to the development of embryos from somatic (non-sexual) tissues (Trigiano and Gray, 2011). However, because the somatic embryos originated from tissues of seedlings in this example, this protocol contradicts itself and derives genetically identical tissues from genetically dissimilar seedlings. Likewise, the growth of zygotic embryos (zygotic referring to the embryo formed from the combination of one sperm egg cell and one egg cell) has the same limitation as expressed previously in regard to seed propagation and the recombination of chromosomes resulting in progeny that are not identical.

A micropropagation study conducted by Ďurkovič (2008) accomplished preliminary research on *C. mas* in tissue culture based on research of tissue culture of *C. florida*. While both species are related, *C. mas* could behave very differently than *C. florida in vitro* which is

common among woody species. Ďurkovič used media types, nutrient concentrations, and hormones reported for use of clonal propagation of *C. florida* in Kaveriappa et al. (1997). Ďurkovič did not evaluate different nutrient salt formulations but instead only WPM (Woody Plant Media) as a result to the findings reported by Kaveriappa et al. (1997) that "WPM proved superior to Murashige and Skoog (MS) as well as Schenk and Hildebrandt (SH) basal media". Ďurkovič (2008) stated that the multiplication rate obtained by growing cultures on 0.7 mg l^{-1} 6-benzylaminopurine (BAP) + 0.05 mg l^{-1} naphthalene acetic acid (NAA) or 1.0 mg l^{-1} BAP + 0.05mg l^{-1} NAA "is a multiplication rate very close to that reported for *C. florida*". This statement assumed that *C. florida* and *C. mas* perform the same in tissue culture. Research has shown, that nutrient salts can have significant effects on propagation rates and can vary by species (Preece, 1995). Examples of research culturing other related *Cornus* spp. include Feng et al. (2009) in which MS nutrient salt formulation was to be preferable to culture *C. canadensis* and Ilczuk and Jacygrad (2016) which found WPM to be the preferable nutrient salt formulation *C. alba*. Li et al. (2015) reported that DKW nutrient salt formulation was utilized for *C. wilsoniana*. Fan and Xiang (2001) show that *C. mas* and *C. kousa* are in separate subgenera (subgen. *Cornus* and subgen. *Syncarpea*, respectively), thus the conclusion that both species may not behave identically in culture may be validated. A further limitation of Ďurkovič's research was the use of only one cultivar. Multiple cultivars should be used to obtain a broader understanding of how the species reacts *in vitro*. Developing a strong root structure is essential for *in vitro* propagation. Ďurkovič and Bukovská (2009) published a protocol for rooting *C. mas in vitro*, however that protocol is based on that used for adventitious rooting of *C. florida*. As stated previously regarding micropropagation, genetic differences between the two subgenera that *C. mas* and *C. florida* (subgen. *Cornus* and subgen. *Syncarpea*, respectively) could cause

differences in how the two species react to the same growing conditions. Plantlets produced *in vitro* can be considered softwood rather than hardwood.

Research Objectives

The objectives for the research for *C. mas* described herein are two-fold: to estimate cold hardiness of selections based on artificial freeze testing and to describe appropriate protocol for clonal propagation of selections. Clonal propagation techniques to be evaluated are grafting and tissue culture (micropropagation).

CHAPTER 2. ARTIFICIAL FREEZE TESTS OF *CORNUS MAS*

Abstract

Cornus mas (Cornelian cherry) is a deciduous shrub or small tree that is native to southeastern Europe and western Asia. It is unique among the Cornaceae (dogwood) family in that the fruit is highly nutritious and contains high amounts of antioxidants and anthocyanins. While this plant has many desirable fruiting and ornamental characteristics, it has seen limited use in North America. Little is known about the cold hardiness of this species being that there has been limited use outside of its native range. This study aims to determine if controlled freezing can be used to analyze potential hardiness of different cultivars to select for northern zone field trials. To do this, five cultivars ('Flava', 'Golden Glory', 'Pyramidalis', 'Spring Glow', and 'Variegata'), one variety (var. *elegantissima*), and two individuals (TS79239 and TS7987) were sampled in February, March, and April and exposed to five freezing temperatures (-16, -24, -32, -40, and -48 °C) using a controlled freezing chamber. Stem, flower bud, and vegetative bud tissue were evaluated for hardiness. Electrical conductivity (EC) was recorded for each tissue sample after exposure to freezing treatment temperatures and heat killed temperature. Regressions suggest that injury indices for selections generally increase with degree day accumulations and temperature treatments.

Introduction

Cornus mas belongs to the Cornaceae family, commonly known as the dogwood family. Related species include many ornamental species such as *Cornus kousa* (Buerger ex Miq.) Hance (Kousa Dogwood) and *Cornus florida* L. (Flowering dogwood). While other members of the family have large, showy flowers, *C. mas* flowers emerge before the leaves and surround the plant in a halo of yellow, comparable to *Forsythia* x'Meadowlark'. Some cultivars have

variegated foliage. *Cornus mas* is unique among its family for having edible and nutritious (as well as ornamental) fruit. In its native range of western Europe and eastern Asia, the fruit is used for many culinary uses including preserves, jams, syrups, soda, and baked goods. Benefits include high antioxidants and anthocyanins (Dirr, 2009). Pest and disease resistance add an additional benefit (Klimenko, 2004).

With the desirable qualities this species exhibits, the species has seen limited use in the North American landscaping industry. *Cornus mas* could have large potential as an ornamental and/or edible landscaping plant, primarily in cold regions where flowering plant selections are limited. With many cultivars of different colors, shapes, and flavors of fruits, this species could add diversity to the American landscape.

With the lack of information on this species relevant to the western hemisphere, cold hardiness has never been defined. Only a few cultivars in certain texts are given an area of hardiness based on observations of only a few individuals (Cappiello and Shadow, 2005). Sources differ on whether *C. mas* belongs in the USDA cold hardiness zone 4 or zone 5. A definitive categorization would help nursery producers, landscapers, and horticulturalists in general planting of *C. mas* in areas that are environmentally suitable for species survival. Research by Karlson et al. (2004) shows that *C. mas* has a different supercooling mechanism in the xylem compared to hardier species such as *C. sericea*, which is considered one of the hardiest *Cornus* species (Dirr, 2009). With the species having a large natural and naturalized range, it can be assumed that a wide range in hardiness is possible.

As stated in Aitken and Adams (1996), "labor intensive assessment may not be feasible for some tree improvement programs, where the need to screen large quantities of progeny to rank for parent trees for cold hardiness." The current experiment testing *C. mas* for cold

hardiness has the intention of ranking genotypes for cold hardiness to determine a range of hardiness for the species rather than for the purpose of identifying parents for breeding cold hardy selections. Aitken and Adams' statement outlines the need for artificial freezing protocols for instances where large scale winter field trials are neither feasible nor appropriate for research objectives. Other species of woody plants have been tested for cold hardiness using various artificial freezing methods including grape (*Vitis vinifera* L.), Douglas-fir (*Pseudostuga menziesii* (Mirb.) Franco), azaleas (*Rhododendron* ssp.), and white spruce (*Picea glauca* (Moench) Voss). In Quamme (1986), grape buds on stem segments were placed in a control freezing chamber and chilled at a steady rate 5 °C/h with thermocouples attached to detect when bud death occurred. The methodology for Mills et al. (2006) was much the same for grape buds, except bud death was detected using thermoelectric modules contained in sensitive Peltier plates. Cooling rate (4 °C/h) was similar to Quamme (1986). It is important to note that Mills et al. (2006) states that while cooling rates as rapid as 10 °C/h have been used in previous studies, this rate is not realistic or representative of freezing events in nature. They further state that a cooling rate of 4 °C/h is equitable to a standard system used in other laboratories. Quamme (1973) describe cold hardiness as the temperature at which 50% of tissue is injured or the temperature at which significant injury is first detected using low temperature exotherms (LTE_{50}). Quamme (1986), Aitken and Adams (1996), and Mills et al. (2006) also use LTEs to describe tissue damage. Väinölä et al., (1997) use a similar format to describe injury called lowest survival temperature (LST) at which ≥50% of the tissue samples survived. The studies performed by Aitken and Adams use artificial freezing to see at which point in the fall (1996) and spring (1997) woody tissues are most susceptible to freeze damage. While Aitken and Adams have similar freezing rates to Quamme (1986) and Mills et al. (2006), used only visual scoring

(qualitative) of freezing damage rather than a quantitative method. Väinölä et al. (1997) used differential thermal analysis to compare azalea flower buds and stem segments using thermocouples and visual ranking of tissue death. Hamilton et al. (2016) reported measuring electrolyte leakage from tissues for measuring the damage at different temperatures. This method is far more efficient than the methods of Aitken and Adams (1996; 1997) and does not require thermocouples as in Quamme (1986).

Howe et al. (2003) reported that woody plants are at greatest risk of cold injury when plants are actively growing, and frost temperatures are less common (i.e. in late spring during bud break). Results suggest that, while early frosts in autumn pose injury risk to plants, spring frosts are of greater risk to plant survival and that spring cold injury is highly dependent on timing of bud break while bud set in autumn has a more variable relationship with cold hardiness (Howe et al., 2003). Hannerz (1999) reported that shoots of plants become highly susceptible to low temperatures during bud break with bud break timing (under genetic control and triggered by environmental factors, i.e. temperature sums or degree days) being useful for cold temperature tolerance screening in woody plant breeding programs. For example, a Swedish breeding program selected for reduced frost risk in Norway spruce (*Picea abies* [L.] H. Karst) (justified by spring frost being a serious threat to survival and quality of spruce stands) (Hannerz, 1999).

Variation in cold damage is exhibited between different populations of woody plant species (Howe et al., 2003). This variation can be found in different parts of native growth ranges for different species. This gives strong evidence to suggest that cold hardiness is a result of natural selection. Howe et al. (2003) suggest that cold adaptation phenotypes are affected by geographical scale among populations and that the natural selection of these phenotypes is strongly associated with climate and geographical factors. For example, when comparing two

Douglas-fir populations, one costal and one inland at higher elevation, the population further from the ocean and exposed to a cooler climate has lower chilling requirements to break bud dormancy. This correlation could be caused by interactions of thermal time (heat sums), chilling requirements, and geographic environments. Another example of environment affecting cold hardiness, would be in regions where shorter growing seasons impact early bud set in autumn. It is suggested that climate factors are more important than geological location when genetic clines are being tested. Climate factors can be expressed as temperature means or minimums, number of frost-free days, or accumulation of degree days. While differences of cold adaptations between populations of like species can be observed, differences in phenotypes can also typically be found within populations. Microclimates, such as understory conditions or exposures on hillsides, may cause highly localized adaptations. Phenology for cold adaptations is suggested to be under moderate to strong genetic control according to Howe et al. (2003). However, the authors state that spring is under stronger genetic control than autumn (with adaptations for autumn acclimation to cold being more tissue-specific). There is also potential for genotype by environmental interactions.

Howe et al. (2003) reported that reproductive tissues of woody plant species (flower buds and related organs) are generally more susceptible to cold injury than vegetative structures. In many of these species, flower bud break occurs before vegetative bud break, and therefore these reproductive structures are exposed and more vulnerable to late spring frosts. Väinölä et al. (1997) reported that flower organ and bud survival during winter is crucial for woody plants that depend on floral display (and subsequent fruit set) as a main ornamental attribute. Väinölä et al. (2003) also suggested that timing of acclimation and deacclimation to cold temperatures differ

between reproductive and vegetative structures. Cold hardiness is a common breeding objective for species in woody plant breeding programs (Howe et al., 2003).

The experiments described herein aim to show at which point in the spring *C. mas* is most vulnerable to cold damage as like Aitken and Adams (1997) while using the methodology of Hamilton et al. (2016).

Materials and Methods

Plant material source

Five *Cornus mas* cultivars ('Flava', 'Golden Glory', 'Pyramidalis', 'Spring Glow', and 'Variegata'), one variety (var. *elegantissima*) sourced from University of Wisconsin-Madison Arboretum (Madison, WI, USA; 43.0416, Long: -89.4311; USDA hardiness zone 5a), and two individuals from the NDSU Dale E. Herman Research Arboretum (Absaraka, ND, USA; Lat: 46.9859, Long: -97.3549; USDA hardiness zone 4a) were used for the controlled artificial freeze tests. The two individuals sourced from Absaraka, ND, TS79239 and TS7987, will be referred to as 'Absaraka-1' and 'Absaraka-2' respectively for the rest of this document. 'Absaraka-1' was obtained from Wayside Gardens (Greenwood, SC, USA) and 'Absaraka-2' was obtained from the University of Washington (Seattle, WA, USA); both were planted at the NDSU Research Arboretum in 1979. The origin of the seed for these two plant accessions is unknown. The selections were exposed to five temperatures (-16, -24, -32, -40, and -48 °C) using a controlled freezing chamber (Tenney TC Series Cycling Test Chamber, Model No. TC20C2.0-A-351-C, Thermal Product Solutions™, New Columbia, PA, USA) as well as a control temperature treatment of 4 °C.

These selections were used based on the genetic diversity expressed as phenotypic differences in foliage type, fruit shape and color. It had been observed beforehand that both

‘Variegata’ and ‘Absaraka-2’ were only marginally hardy for their respective locations, showing dieback after each winter (T. West and D. Stevens, personal correspondence). The selections ‘Variegata’ and var. *elegantissima* are variegated and tricolor (white, green, and pink) foliage types. The cultivar ‘Flava’ was the only yellow-fruited selection used in the experiments. ‘Variegata’ is a known fruitless cultivar and ‘Absaraka-2’ has never produced fruit but is not proven as fruitless. The rest of the selections used have some shade of red fruit and are of a typical green foliage type. It is known that var. *elegantissima* was selected in Germany, however the other selections tested have no record of seed origin. ‘Spring Glow’ was selected in Raleigh, NC, USA and ‘Golden Glory’ was selected in Chicago, IL, USA with no record of seed origin.

Artificial freezing protocol

Artificial freeze tests were conducted as described in Hamilton et al. (2016). Stems were cut into 5±1mm segments and placed two per 55.75 x 16mm sample vial (Peti-Vial, Product No. 100491-992, VWR International, LLC., Radnor, PA, USA) with 0.2 ml distilled water and silver iodide (AgI, molecular weight of 234.77; Product No. 204404, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Vegetative buds and flower buds were placed singly in sample vials for the selections. There were three replicates for each tissue type (stem, vegetative bud, and flower bud) of each selection at each temperature (-16, -24, -32, -40, -48 °C). Samples were refrigerated overnight at 4 °C to bring solutions to equilibrium. Samples were placed in controlled freezer at 4 °C and lowered at a rate of 4 °C/h and held at each of the temperature treatments (-16, -24, -32, -40, -48 °C) for one hour prior to removal. A rate of 4 °C/h is standard for other experiments as stated in Mills et al. (2006). Control samples of each selection and tissue type were placed in sample vials with distilled water and silver iodide and stored at 4 °C while other samples were exposed to the freezing temperature treatments. Control samples were used to compare against

different temperate treatments and to calculate injury index of the other temperature treatments. After freezing, an additional 3.5 ml of distilled water was added to each sample vial and all samples returned to the refrigerator at 4 °C for 24 h. The control samples also received an additional 3.5 ml distilled water and were returned to 4 °C for 24 h. Samples were shaken for one hour before the initial electrolytic conductivity (EC) measurement (Fisherbrand™ Traceable™ Expanded Range Conductivity Meter; Product No. 15-077-977; Thermo Fischer Scientific, Inc., Waltham, MA, USA). Samples were then heat killed for two and a half hours at 95 °C (as suggested by J. Hamilton; personal correspondence), shaken again for one hour, and EC was measured a final time. The heat kill procedure used for *C. mas* was a modification of the protocol described by Hamilton et al. (2016), which utilized pine needles instead of *C. mas* stem and flower bud tissues. *C. mas* tissues have more lignin and cellulose content resulting in less cellular damage as compared to pine needles after one hour of a 95 °C heat kill to measure an accurate damage ratio.

Index of injury (*I*t) was calculated in the following equation from Hamilton et al. (2016):

$$I\mathrm{t} = \frac{100(R_t - R_o)}{1 - R_o}$$

where Rt = Lt/Lk and Ro = Lo/Ld, determined from the ratio of conductivity between frozen samples preceding (Lt) and following heat kill (Lk) and between unfrozen control samples (those kept at a constant of 4 °C) preceding (Lo) and following heat kill (Ld). Any negative index of injury levels calculated with this formula were changed to a value of 0 during the statistical analyses being that it is physically impossible to sustain negative frost damage. Likewise, any values above 100 were changed to 100 during data analysis as it is impossible to have an injury index above 100.

This experiment was repeated at different time periods (months): February 15, March 21, and April 20 in 2018. Fresh tissue samples were taken 24 h prior to being processed and introduced to the vials. Tissue at these dates was dormant (February 15), coming out of dormancy (March 21), and finally breaking flower bud (April 20) (Figure 2-1). The reason why runs were conducted during these times was to see if and how tissue is damaged by cold at different stages of dormancy. It should be noted that the cultivar 'Variegata' was only tested during dormancy in February since the amount of tissue available for the experiments was limited.

Degree day data (above 5 °C) were collected from ClimateNA_MAP (Centre for Forest Conservation Genetics, 2018) using the decade 2001-2010 to calculate monthly degree day above 5 °C (DD5) averages for Feb, March and April. Latitudes and longitudes for locations are as follows: Lat: 46.9859, Long: -97.3549 for the NDSU Dale E. Herman Research Arboretum and Lat: 43.0416, Long: -89.4311 for University of Wisconsin-Madison Arboretum.

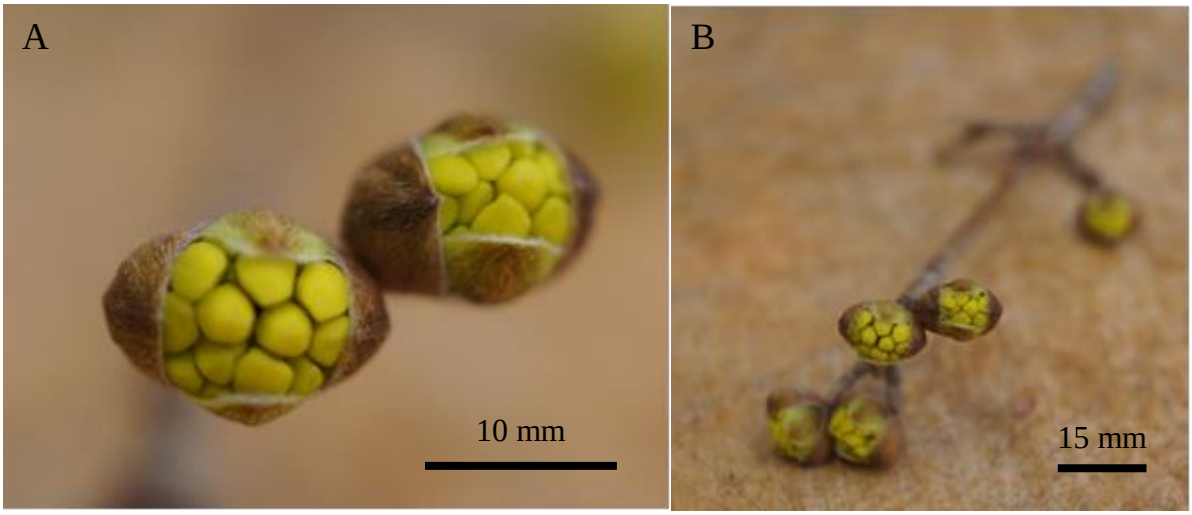

Figure 2-1. Buds from *Cornus mas* used for the artificial freeze tests were taken at three different times, at which physiological changes were occurring in response to increasing photoperiod and temperatures. The pictures above were taken on April 19, which was the final run of the experiment. The flower bud is opening, and the flower structures are exposed (close-up view in A; overall view in B).

Experimental Design

The experiment was arranged as completely random designs (CRD) and conducted twice. Two treatments; temperature (-16, -24, -32, -40, -48 °C) and tissue type (stem, vegetative bud, and flower bud) were combined in a 5x3 factorial with three replicates per treatment. The experiment was conducted in three separate months (February, March, and April in 2018. Tissue damage was recorded using the index of injury equation found in Hamilton et al. (2016). Regressions were made using R (The R Foundation, 2019). Data were analyzed using the General Linear Model (GLM) of (SAS 9.3; SAS Institute Inc., 2018). Repeated measures were not used as samples from Madison, WI came from several individuals of the same accessions with sample pooled together.

Results and Discussion

Regressions were compiled using degree days above 5 °C on the X-axis and injury index (as a percentage) is represented on the Y-axis. Slopes and Y-intercepts for regressions in these figures can be found in Appendices C, D, and E, respectively. The selection 'Variegata' is represented in the legends, however because sampling material for this selection was limited to only one sampling period, regressions and comparisons to other selections cannot be made for 'Variegata'. Regressions show that damage of samples caused by cold generally increases both with increasing degree days and decreasing cold treatments. There was a significant effect with temperature and DD5 on each of the three different tissue types. (Appendix A). For all tissue types the effects of degree day accumulations above 5 °C (DD5), temperature, and the interaction between these two effects show significance (Appendix B). This would suggest that as temperatures increase in springtime and degree days accumulate, the susceptibility of cold damage to *C. mas* also increases. The results showing increased susceptibility to cold at

increasing degree days match the assertations of Howe et al. (2003) that bud break correlates with an increased susceptibility to cold damage during bud break.

While regressions have general trends of all tissues having greater damage at colder temperature treatments and greater accumulations of degree days, regressions suggest that damage was significantly different for the tissues being sampled (with stems exhibiting greater damage than flower buds or vegetative buds). This is contrary to Howe et al. (2003) which states that reproductive tissues are more susceptible to cold damage than vegetative structures (such as stems). Results from Väinölä et al. (1997) showed that florets (Rhododendron) were at greater risk to cold injury than stem segments. The discrepancies between previous publications and the results obtained here may indicate the protocol for *C. mas* may require a greater "heat kill" time to extract all electrolytes from the samples into the water solution for a more accurate injury index.

Figure 2-5 only shows the change in rate for the different tissues and selection sampled, not the change in injury index. Therefore, differences in slopes are observed in this figure only. For example, in the graph representing stem, it can be seen that 'Absaraka-1' had a greater rate of change than 'Absaraka-2'. In graphs representing flower buds and vegetative buds, 'Absaraka-2' has a decreasing rate of change of cold tolerance in relation to degree days. 'Absaraka-2' is the only selection showing this decrease in slope. Other selections were generally consistent to show increasing rates of change in response to degree days above 5 °C and colder temperature treatments. This would be expected as tissues deharden in the spring and become more susceptible as stated in Hannerz (1999) and Howe et al. (2003).

Appendix B shows that the interactions of selection by degree day accumulation and temperature treatment by degree day accumulations are significant. This can be seen in

regressions where injury of relative selections generally increases with increasing degree day accumulations. Selection which represents the different cultivars and variety had a significant effect on cold temperature injury (Appendix B).

Comparisons of the Absaraka, ND accessions can be made with the selections sampled from Madison, WI in the regressions at relative degree days above 5 °C. In the regression graphs representing stem samples, 'Flava' shows consistently more damage than other selections for all temperature treatments. 'Spring Glow' shows less cold injury than other selections for stem samples (Figure 2-2). For flower bud samples, 'Spring Glow' shows more cold injury than other selections for temperature treatments -32, -40, and -48 °C while 'Flava' and 'Pyramidalis' show less cold injury (Figure 2-3). For vegetative buds, 'Spring Glow' and var. elegantissima are generally consistent with having injury indices higher than other selections, with the exception for the -32 °C temperature treatment in which 'Golden Glory' has the highest cold injury. 'Pyramidalis' and the Absaraka accessions show less cold injury than other selections for vegetative bud samples (Figure 2-4). 'Absaraka-1' and 'Absaraka-2' generally have decreasing cold injury as shown by their regressions, with the exception of stem samples at -40 and -48 °C (Figure 2-2). However, the accessions sampled from Absaraka, ND are generally on the low end of the cold injury spectrum for flower and vegetative buds. The inconsistencies between tissue type regressions may indicate a need for changes to be made to the freezing protocol as previously mentioned.

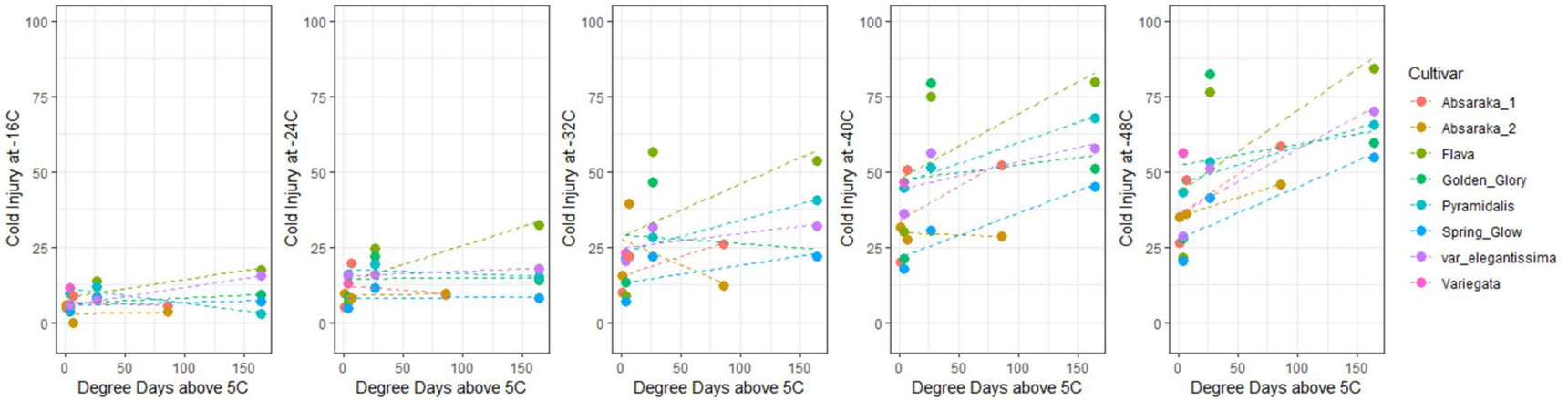

Figure 2-2. Stem tissue regressions; each graph represents one temperature treatment.

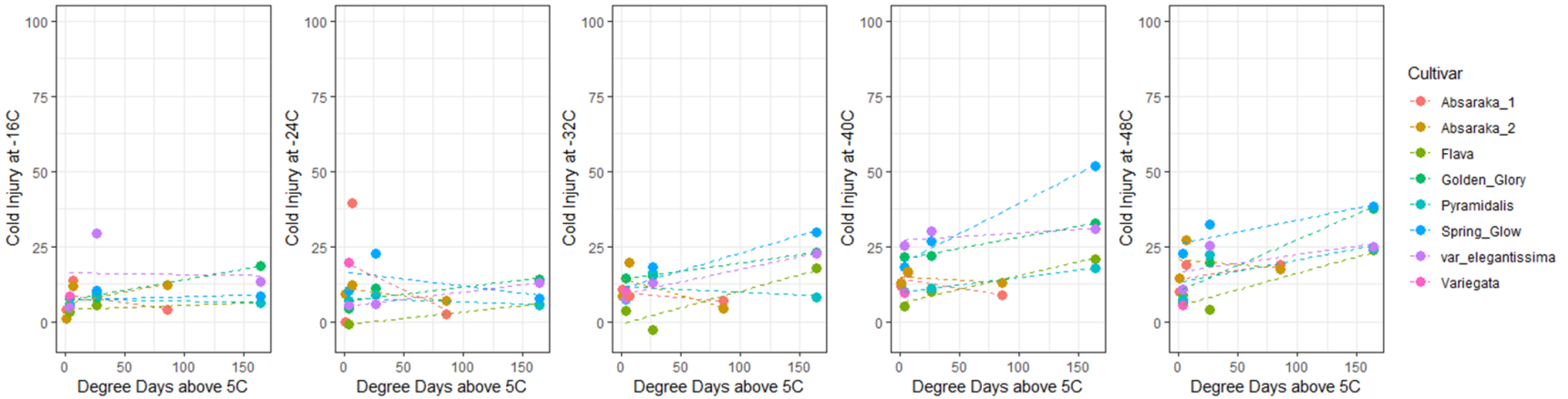

Figure 2-3. Flower bud regressions each graph represents one temperature treatment.

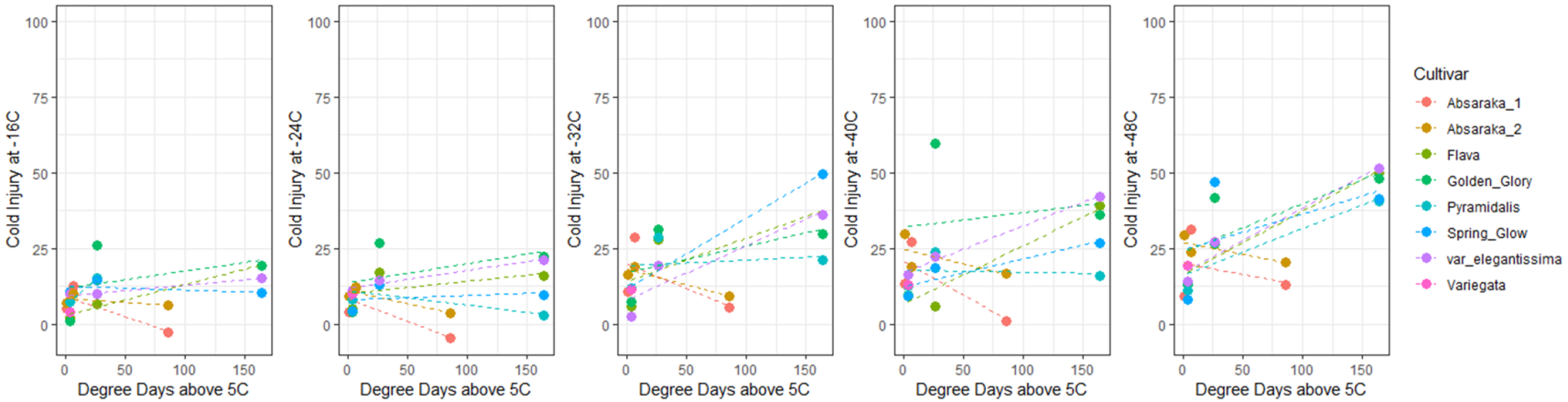

Figure 2-4. Vegetative bud regressions each graph represents one temperature treatment.

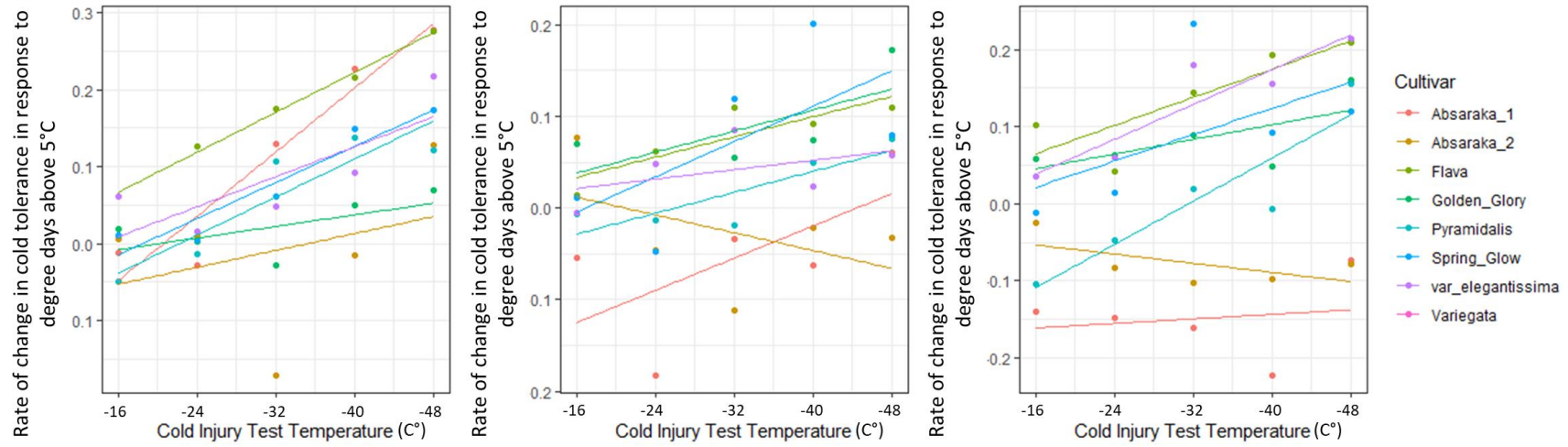

Figure 2-5. Rate of change for tissue samples (left: stem, middle: flower bud, right: vegetative bud)

Conclusions

Adaptations to cold hardiness are complex and influenced by both genetic and environmental growing conditions (Howe et al., 2003). While it is difficult to say which accession tested is the hardiest, results give evidence that accessions are indeed different in the amount of damage or injury that is caused to different tissues with decreasing temperature treatments. More importantly, results show that samples became more susceptible to cold injury as more degree days above 5°C accumulated at sampling sites. These observations are expected and, as explained by Hannerz (1999) and Howe et al. (2003), give credence to woody plant tissues becoming more susceptible and "less hardy" to late spring frosts as both flower buds and vegetative buds begin to break.

Results from artificial freeze tests and regressions compiled using this data suggest that plant tissues sustain more damage with colder temperatures as well as with increasing degree day accumulations, as would be expected based on regression analysis. Observations of different accessions at the two locations suggest that some selections sustained more cold damage than others. For example, at the University of Wisconsin-Madison Arboretum, the selection 'Variegata' sustained more cold damage than its counterparts. Likewise, at the NDSU Dale E. Herman Research Arboretum, 'Absaraka-1' sustained less cold injury than 'Absaraka-2'. These observations suggest there could be differing cold adaptations between selections of *C. mas* as well as the populations they had originated from. With a large native range, it is highly possible localized natural selection occurred in native populations of the species, which could in turn be reflected in progeny accessions originating from these native populations. Data from the artificial freeze tests show higher damage in stem tissue samples compared to flower buds or vegetative buds. The cause of this is unknown as previous publications suggest the opposite; reproductive

structures are more susceptible to cold damage prior to and during break (Howe et al, 2003; Väinölä et al. 1997).

CHAPTER 3. GRAFTING *CORNUS MAS*

Abstract

Cornus mas (L.) is a dogwood native to eastern Europe. While it has qualities that make it a promising ediscaping plant, it is a generally underutilized species outside of its native range. A major limitation in a species or cultivar to be sold to consumers is the initial propagation required to produce genetically identical plants. Many woody plant species are propagated using grafting or budding onto a rootstock to maintain genetic homogeneity. Two selections were used in the study described and performed herein; 'Pyramidalis' and an individual (TS79239) referred to as 'Absaraka-1'. The graft types used were the side graft and the chip bud graft. Results indicated that both selection and graft type had significant effects on success and survival of grafted scions. [1]

Introduction

Grafting and budding have been used for over 2000 years and is still used to this day for multiple purposes (i.e. propagating plants that are difficult to produce by other means; selecting benefits not apparent in the scion donor plant, like disease resistance or dwarf/weeping forms; propagation of cultivars or clones) (Dirr and Heuser, 1987). Grafting can be summarized as the process of two plants, or plant parts, creating a bond to grow together as one plant. In commercial propagation, this occurs as a scion (donor material consisting of one or more bud) is bonded to a rootstock (which becomes the root system for the final grafted plant). To graft two

[1] Information from this abstract may have been previously published in an online supplement of the journal HortScience, Volume 53(9S), as presented in a poster at the American Society for Horticultural Science 2018 conference. Meredith Swanson had primary responsibility for writing said abstract, as well for as conducting experimental protocol, taking measurements, and developing conclusions for the experiment described. Todd West served as proofreader for previous works coauthored with Meredith Swanson and published in HortScience, Volume 53(9S).

plants, cuts are made on both the scion and rootstock to expose the cambium, or vascular tissue, of both plants. The cambium of the scion is matched to that of the rootstock. The two are secured with a wrapping material (i.e. rubber budding strips, Parafilm M, twine, adhesive tape, etc.). If the securing of the scion to the rootstock is successful, a bond will form between the two. This bond is formed in a process of callusing (formation of dedifferentiated cells) of both scion and rootstock, differentiation of cells in the calli to form meristem and vessels, and healing (lignification) of calli cells (Dirr and Heuser, 1987). In the process of bonding a graft, it is important to create a barrier against outside water (as in the case of Parafilm M or plastic). If water comes between the cambial layers of the scion and rootstock, the two will fail to form a bond and the graft will fail. A large multitude of grafting types exist and can be generalized into two categories: grafting (use of two or more buds per scion) and budding (scion consisting of one bud on a slice of bark). For the purposes of this experiment, one grafting method and one budding method will be described: side grafting and chip budding.

For a side graft, a slice is made into the side of the rootstock, near to the soil, leaving a flap of bark on the side of the cut. A scion is prepared by making long, straight cut on one side and a smaller cut on the opposite side (creating a wedge at the base of the scion). The scion is then placed between the exposed cambium and the bark flap and secured (Dirr and Heuser, 1987).

For chip budding, a short and shallow cut is made into the side of the rootstock at an acute angle. A long and shallow secondary cut is made into the same side of the rootstock above the first cut to expose the cambium and remove a section of bark. A scion is cut in the same way to remove a strip of bark containing a single bud (the “chip”) from donor material. The cambial layers of rootstock and scion are matched and secured (Dirr and Heuser, 1987).

A novel species such as *Cornus mas* requires a viable clonal propagation method for cultivars to be produced and distributed to consumers. Grafting is a common method used for clonal propagation of woody ornamental cultivars. Dirr and Heuser (1987) reported that there are no mentions of grafting methods found in the literature for *C. mas*. There are several cultivars commercially available in the U.S. and they are listed as grafted clones. It is not common practice to give detailed information on propagation protocols within the commercial industry and this information is rarely published or made available because of potential financial losses from other competitive nurseries. The NDSU Woody Plant Improvement Program utilized grafting to propagate cultivars acquired from various sources but no comparison of grafting types and methods were initially conducted. While not all graft unions were successful, the method has proven to be useful in maintaining cultivar populations.

Since Dirr and Heuser's assessment of propagation techniques in 1987, grafting success has been reported by Bijelić et al. (2016). Bijelić et al. (2016) evaluated two grafting types, summer bud grafting (August of 2011 and 2012) and spring whip grafting (April of 2011 and 2012) with only a single graft type evaluated during each seasonal period. All of these graft evaluations were conducted in outdoor field conditions at a nursery in Novi Sad, Serbia (Bijelić et al., 2016). Field grafting can be difficult and is not always optimal for grafting success. Temperature changes, soil conditions, and water amounts could all be variable in a situation like this. Rainwater could corrupt graft unions by infiltrating between the scion (donor section) and the rootstock easier than manual watering in a greenhouse where care is taken to wet only the soil and not the plant tissues. In the article graft success, scion length and diameter, fruit weight, and root length were recorded. Bijelić et al. (2016) reported "very satisfactory" results with the highest success rate of summer bud grafting being 83.62% with an average of 69.38% in that

same grafting period. The authors concluded that grafting *C. mas* "produces quality planting material, can be recommended for mass production, and is suitable for production of the first-class planting material". The authors further report that the findings of such a stable propagation method caused *C. mas* to gain a better foothold in Serbia. After this paper was published, commercial orchards were planted, and production steered away from the traditional wild gathering practices "which, until recently, grew only in forests" (Bijelić et al., 2016). The objective of the experiment is to compare different grafting methods for clonal propagation of *C. mas*.

Materials and Methods

This study was conducted in 2017 in the south Lord and Burnham Greenhouses at the North Dakota State University campus (Fargo, ND, USA; Lat: 46.8921, Long: -96.8053). Scions for the study were from two separate locations: at the NDSU Dale E. Herman Research Arboretum (Absaraka, ND, USA; Lat: 46.9859, Long: -97.3549) and the University of Wisconsin-Madison Arboretum (Madison, WI, USA; Lat: 43.0416, Long: -89.4311). The selections TS79239 (referred subsequently to as 'Absaraka-1') and 'Pyramidalis' were collected from these locations respectively. The experiments were conducted in April and August 2017 to observe seasonal differences in graft success rate. Scions were taken in the spring before buds had broken dormancy for the April experiment and scions were taken in late summer for the August experiment. Two-year old *C. mas* rootstocks were used for grafting experiments. This study tested two different grafting types: side grafting and chip budding (Figure 3-1). A third graft type, T-budding, was attempted. However, at the time of the April experiment the bark on the rootstocks was not slipping enough to facilitate and for this reason T-budding does not appear in any results. Care was taken to ensure the cambial tissues were in alignment to have the

best chance for a successful union during the grafting process. Grafts were secured to the rootstocks with budding rubbers and then wrapped with Parafilm M® (Bemis Company, Inc., Neenah, WI, USA) to prevent water from corrupting the graft union. The grafts were kept in a greenhouse and watered daily, taking care to not get the graft union wet.

For the spring experiments, the first and second runs were grafted April 13 and 19 respectively. The rootstocks of grafted plants were cut back to a few inches above the graft union on May 5 to shock the plant in an attempt to force the grafted bud to open. Parafilm M® was removed to observe for graft success on May 15. On May 25, the rootstock was cut back just above the graft union.

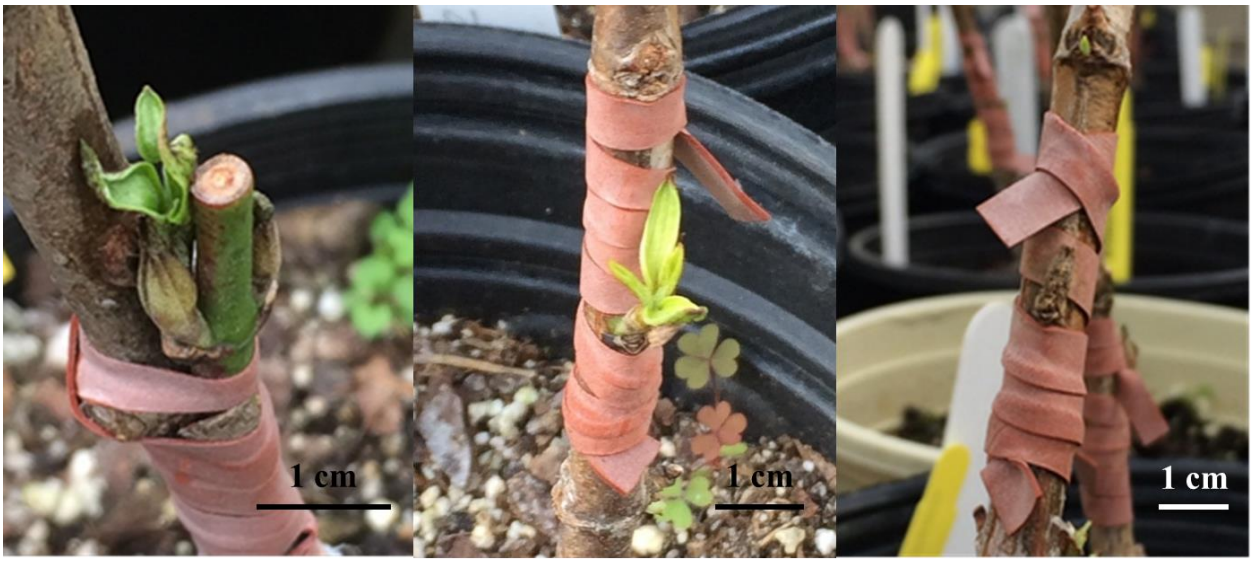

Figure 3-1. Three different grafting types were evaluated, side grafting (left), bud grafting (center) and t-budding (not shown). The picture at right exhibits a failed graft where the bud has turned brown and never formed a union to the rootstock.

The experiment was repeated August 17 and 18 at the same location in an attempt to evaluate effects of spring versus late summer grafting success. Fresh buds were taken from parental plants as stem segments with leaves excised. No data was taken from the August experiment as all grafts failed. Buds, which had been harvested from the plant selections as fresh

material (in August of the same year) rather than using material from the spring experiment, were observed to be brown and dead when the parafilm was removed. Therefore, data analysis was not performed for the late summer portion of the experiments.

Experimental Design

These experiments were conducted as an CRD (Completely Randomized Design) factorial consisting of selection by graft type (chip bud and side graft) and cultivar ('Absaraka-1' and 'Pyramidalis') with five replicates of each treatment with the entire experiment being repeated twice. Grafting success and length of scion were recorded. Data were analyzed using SAS 9.3 (SAS Institute Inc., 2018) as a GLM (General Linear Model). Means were analyzed based on a means separation using a student's T-test.

Results and Discussion

There was a significant 2-way interaction between cultivar and graft type on survival rate of grafted scions (Appendix F; Figure 3-2). Side grafts of 'Absaraka' and 'Pyramidalis' had higher survival rates, both cultivars with 100% survival, than that of 'Pyramidalis' chip bud (30% survival). Chip bud grafts of 'Absaraka' were not significantly different from either cultivar or side graft 2-way interaction. These results suggest that utilizing the side grafting method in spring is superior to graft survival as compared to the 60.38% survival with bud grafting as reported by Bijelić et al. (2016). This increase in success rate allows for grafting to be a commercially viable propagation option for developing clonal populations. Buds were recorded as dead if they had turned brown and dry and had no callused graft union to the rootstock. Dead buds were only used to determine survival rate for the experiment and were not included in analysis of scion length, hence type III mean square was used for the analysis of data.

There were no significant 2-way interactions between cultivar and graft type on scion shoot length. There was a significant difference between cultivars on scion shoot length. Shoots resulting from grafted scions of the 'Absaraka' selection were significantly longer (38.7 cm) on average than the 'Pyramidalis' grafts (25.8 cm); graft type showed non-significance (Appendix H; Table 3-1). The difference in graft scion lengths may be a result of genotype differences between the two selections. Bijelić et al. (2016) stated that genotype had a significant effect on both success and other parameters measured in their study, including plant height (in their case, the accession R1 had significantly more lateral growth than the other accessions used in that study). Bijelić et al. (2016) did not specify if plant height was measured from the soil level of the plant or from the graft union. Scion length (as measured and discussed previously) is defined as length of shoots originating at the graft union.

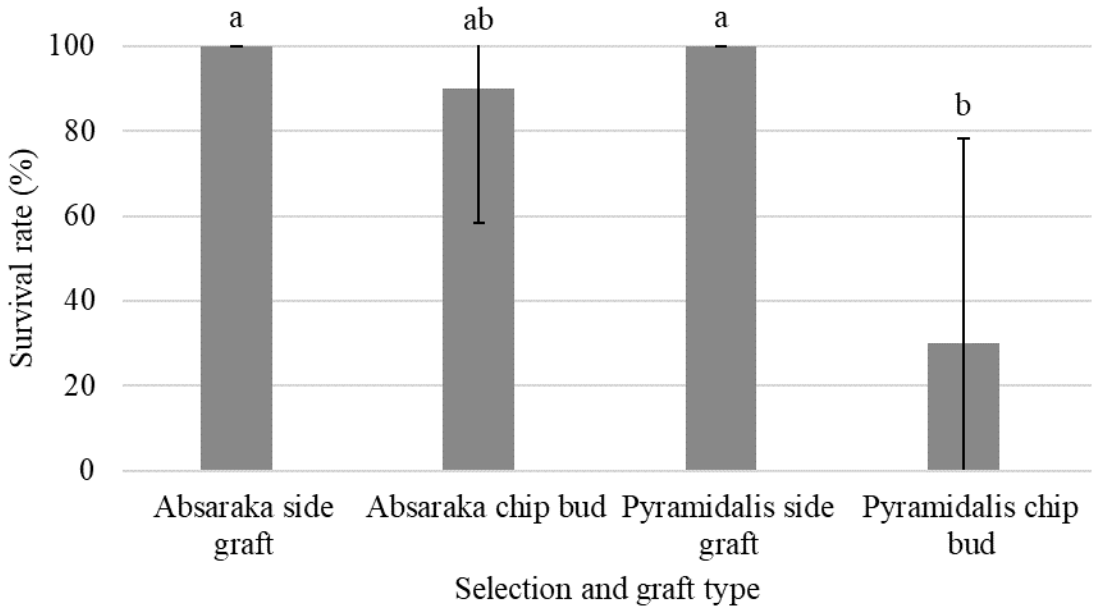

Figure 3-2. Survival rate of different graft methods. Means followed by the same lower-case letter are not significantly different ($\alpha<0.05$); treatment n=10.

Table 3-1. Length of shoots from scion of different *Cornus mas* selections

Selection	Scion shoot length (cm)*
Absaraka	38.7 a
Pyramidalis	25.8 b

* Means followed by the same lower-case letter are not significantly different ($\alpha<0.05$)

The late summer (August 2017) experiment resulted in all buds failing to form a graft union to the rootstock. Subsequently, all buds died. It is for this reason the late summer portion of the study was excluded from data analysis. The findings of the late summer grafting experiment contradict results by Bijelić et al. (2016) that bud grafting in August will result in successful grafts. The experiment conducted herein utilized the individual genotype identified as 'Absaraka' and the cultivar 'Pyramidalis'. Differences used in genotypes and selections used may account for, in part, differences in the results of Bijelić et al. (2016) and this study. Genotypes used in Bijelić et al. (2016) were identified as accessions CPC16, APRANI, BACKA, RI, and PPCI. It can also be noted that the average bud grafting success (average of all bud grafted experimental individuals, regardless of selection) in this study was 60% in spring compared (Figure 3-2) to Bijelić et al.'s average of 69.38% for bud grafting in August. Bijelić et al. (2016) only tested bud grafting in summer, therefore there is no spring percentage from their experiment to compare to. Ercýslý (2004) states *C. mas* cultivars in Turkey are grafted, but do not indicate grafting type or success rates for comparison. Klymenko et al. (2017) reported that the "best time for dogwood budding is from late July to end of August" and that "spring grafting (April) is less effective than in summer". This contradicts the results from this study where it was observed that spring grafting is more effective than summer grafting for *C. mas*.

Table 3-2. Survival rates of two grafting types

Graft type	Mean survival (%)*
Side graft	100a
Bud graft	60b

* Means followed by the same lower-case letter are not significantly different ($\alpha<0.05$)

Other sources for related Cornus species likewise give no results for spring grafting. Dirr and Heuser (1987) state that *C. florida* can be budded in late summer (late July to early September) but do not report success percentages. Like Bijelić et al. (2016), Dirr and Heuser (1987) do not address spring budding. Dirr (2009) states both *C. florida* and *C. kousa* cultivars are "most often propagated by bud grafting in summer"; success rates are not reported. Kaveriappa et al. (1996) reported that *C. florida* is typically "propagated by grafting buds onto native rootstock" without citing preferred season or grafting technique.

Conclusion

These experiments show that while side grafting and chip buds are both valid means of grafting *C. mas*, side grafting can be more successful for survival rate than chip budding to produce clones especially with the cultivar 'Pyramidalis' in spring. Side grafting is preferable to single bud grafting not only for its potential improved success rate, but also for the fact that two buds are present with the scion graft material as a result of the specie's opposite leaf arrangement which provides a secondary bud in case one fails. Bud grafting may be a viable production method as well considering the average success rate across the selections used was 60%. Other sources state that bud grafting is preferable in summer rather than spring for *Cornus* species (Klymenko et al., 2017; Dirr and Heuser, 1987; Dirr, 2009), however no grafts (side or bud) survived summer grafting.

CHAPTER 4. MICROPROPAGATION OF *CORNUS MAS*

Abstract

The use of plant tissue culture, specifically micropropagation, for rapid multiplication of ornamental plants has become one of the fundamental propagation methods for the commercial nursery trade. *Cornus mas* L. (Cornelian cherry) is an underutilized ornamental landscape plant that has promising qualities but relatively little use outside of its native range. The objective of this study was to evaluate the effects of three different nutrient salt formulation (Murashige and Skoog, MS; Woody Plant Medium, WPM; and Long and Preece, LP) on micropropagation of *C. mas*. These three nutrient salt formulations were compared in a factorial experiment with two plant growth regulators (PGRs): 6-benzylaminopurine (BA) and 1-naphthaleneacetic acid (NAA). Data was analyzed from the third subculture (after 18 weeks of nodal segments being *in vitro*). This was because data suggested nodal segments had stabilized to *in vitro* conditions and data was more consistent.[2]

Introduction

There are many benefits of utilizing plant tissue culture, specifically micropropagation, to produce clones of a cultivar or selection of a species. Micropropagation produces uniform, disease-free, and vigorous plants. Unlike grafting or cutting propagation, micropropagation takes up relatively little space and has increased comparative propagation rates. It is for these reasons that micropropagation has become popular in clonal propagation. To date, there are two

[2] Information from this abstract may have been previously published in an online supplement of the journal HortScience, Volume 53(9S), as presented in a poster at the American Society for Horticultural Science 2018 conference. Meredith Swanson had primary responsibility for writing said abstract, as well for as conducting experimental protocol, taking measurements, and developing conclusions for the experiment described. Todd West served as proofreader for previous works coauthored with Meredith Swanson and published in HortScience, Volume 53(9S).

publications of *Cornus mas* micropropagation (Ďurkovič, 2008; Ďurkovič and Bukovská, 2009). Ďurkovič (2008) successfully initiated the cultivar 'Macrocarpa' into tissue culture for their experiments and defined a protocol for shoot proliferation. Ďurkovič and Bukovská (2009) expanded on the previous work of Ďurkovič (2008) to define an *in vitro* adventitious rooting protocol. While these papers did develop a micropropagation protocol for *C. mas*, the protocols from Ďurkovič (2008) and Ďurkovič and Bukovská (2009) were based solely on a protocol for *C. florida* developed by Kaveriappa et al. (1997). The species *C. mas* and *C. florida* are related species being in the same genus. However, Fan and Xiang (2001) showed that these two species are in separate genetic branches (clade) within the genera: the cornelian cherries and big-bracted dogwoods, respectively. These two clades are cited as being sister groups, in that they both have red fruits rather than the white or blue fruits found in other dogwood species (*C. racemosa, C. walterii, C. controversa, C. oblonga*). However, *C. mas* and *C. florida* diverged in their evolutionary history and therefore it is incorrect to assume that the two species behave the same under the same *in vitro* conditions. Ďurkovič (2008) and Ďurkovič and Bukovská (2009) only utilized a single nutrient salt formation, Woody Plant Medium (WPM; Lloyd and McCown, 1980), which was taken from a protocol from Kaveriappa et al. (1997) for *C. florida*. Both reports did not properly evaluate different nutrient salt formulations and the impact this could have on propagation of *C. mas*.

Many different formulations of nutrient salts have been developed for tissue culture and are based on the original Murashige and Skoog (MS medium) which utilized tobacco (*Nicotiana tabacum* L.) (Murashige and Skoog, 1962). The MS nutrient salt formulation is the standard that all other nutrient salt media formulations are based from. Most published micropropagation experiments utilize one nutrient salt formulation, often MS or in the case of woody plants, WPM,

and then only alter the type and concentration of PGRs present in the evaluation medium. Among the many different nutrient salt formulation types, three were chosen for this current experiment: MS, Long and Preece (LP) (Long et al., 1995), and WPM based on the concentration salt levels. MS formulation is considered “high”, WPM being “low”, and LP being an intermediate between MS and WPM (Preece et al., 1995). It was desired for a range of nutrient salt concentrations to be used because, as Preece et al. (1995) reported that it is desirable to evaluate a range of nutrient salt concentrations because when nutrient level and balance are correct, explants are in less stress and performance is drastically improved. The number of adventitious shoots produced as well as the overall health of the cultures (i.e., the exhibition of hyperhydric tissues) can be greatly altered by the use of optimal nutrient concentrations in the tissue culture medium (Preece, 1995). It is recommended that plant tissue culture medium components be evaluated for a plant species including different nutrient salt formulations, PGRs and concentrations and not just based solely on other plant species.

Objectives of this experiment were to evaluate three different plant tissue culture nutrient salt formulations, PGRs and concentrations and the effects on micropropagation of *C. mas*.

Materials and Methods

In February 2017, stems were taken from one-year old grafted material in the greenhouse that was going dormant. Stems were cut into nodal segments (15±3mm in size) with each segment consisting of stem tissue and two axillary buds and disinfected in 70% ethyl alcohol for two minutes. The alcohol was decanted in a sterile laminar flow hood. Nodal segments were transferred to a vessel with 20 g/L sodium dichloroisocyanurate dihydrate (Product No. 218928 Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) with a drop of Tween® 20 (polyoxyethlenesorbitan monolaurate, Product No. P-720, Phytotechnology Laboratories,

Lenexa, KS, USA) for twenty minutes. The solution was decanted in the hood again and rinsed with autoclaved distilled water three times for a minute. The nodal segments were initiated in 25x150 mm glass test vials on MS (Murashige and Skoog) medium containing 30 g/L sucrose and 2.0 µM 6-benzylaminopurine (BA, Product No. B3408, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), solidified with 7 g/L agar (Product No. A111, Phytotechnology Laboratories, Lenexa, KS, USA), with pH adjusted to 5.8. The pH was adjusted with 1N potassium hydroxide (KOH) and/or 1N hydrochloric acid (HCl) before media was autoclaved. A dark exudate was observed as described in Ďurkovič (2008) and cuttings were transferred to new media after ten days and was observed in initiated cultures at North Dakota State University as well (Figure 4-1).

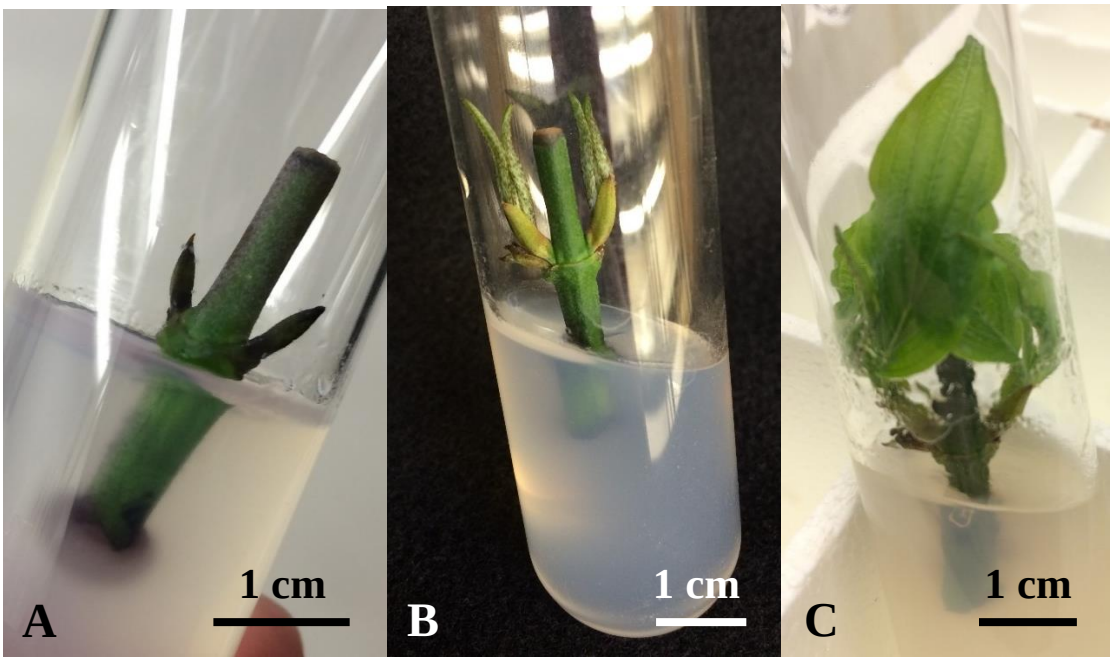

Figure 4-1. Initiated cutting exudes a dark compound at the base of the cutting (A). Buds of initiated cutting break and leaves begin to emerge (B). Shoots begin to elongate from initiated cutting (C).

Nodal segments were grown on an altered WPM (Woody Plant Media), suggested by Ďurkovič (2008), supplemented with 20 g/L sucrose, 6.0 g/L agar, 0.7 mg/L BA (equivalent to

3.11 µM) and 0.05 mg/L 1-naphthaleneacetic acid, NAA; equivalent to 0.27 µM; Product No. N600, Phytotechnology Laboratories, Lenexa, KS, USA), and adjusted to a pH between 5.6 and 5.7. Nodal segments were grown on this medium for 6 weeks to increase culture numbers to be utilized for experimentation. After the initial 6 weeks of culturing, it was observed that the medium from Ďurkovič was not optimal for micropropagating this species. The resulting growth produced leaves that were slender, water-soaked, and translucent (exhibiting hyperhydricity; Figure 4-2). While the initiated cultures were multiplying, the overall condition of the cultures was not optimal. The nodal segments were then grown on LP medium with 30 g/L sucrose, 2.0 µM BA, and 0.5 NAA, solidified with 7.0 g/L agar, and adjusted to a pH of 5.8 for multiplication (Figure 4-3). Cultures were subcultured every six weeks by dividing shoots into nodal segments that were 10±2mm in length to be transferred to new media. Temperature was maintained at 25±3°C and light was provided by cool white fluorescent lamps at a 16 h photoperiod with a photon flux of about 40 µmol m^{-2} s^{-1}.

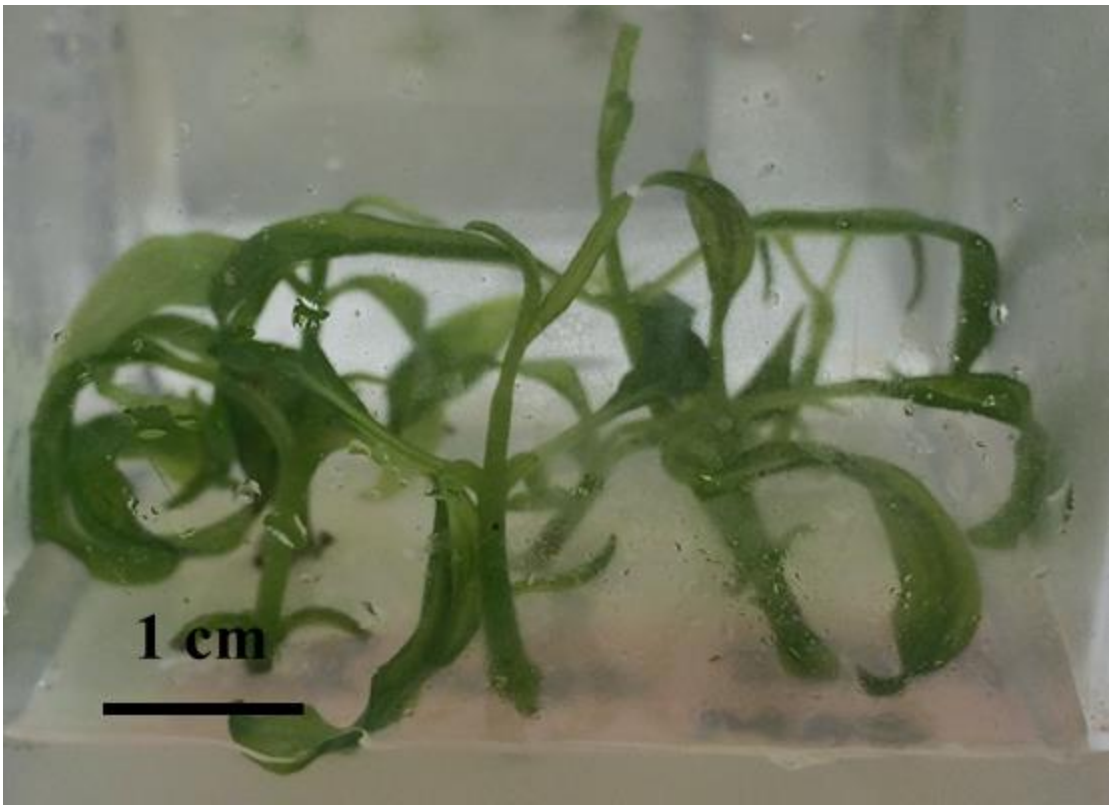

Figure 4-2. *Cornus mas* cultures grown on modified WPM media exhibited hyperhydricity.

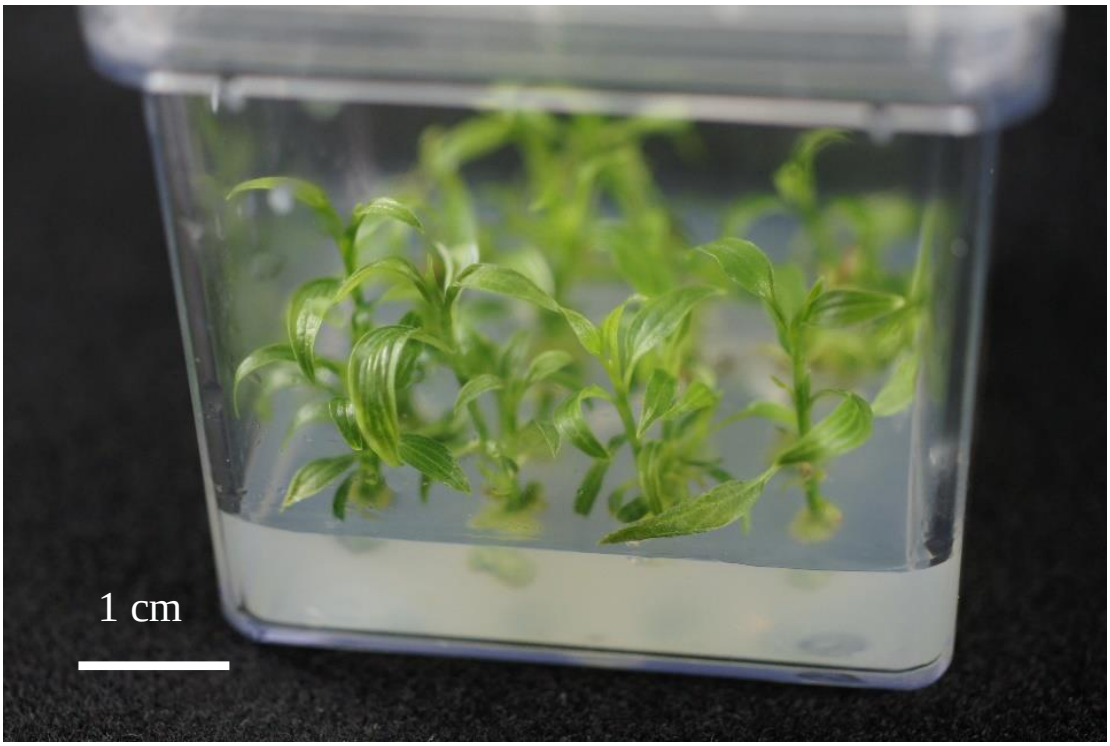

Figure 4-3. Cultures used for the factorial experiment after being grown on modified LP medium.

The cultivar ‘Schonbrunner Gourmet’ was used for formal experiments, as this was the selection with the most available sample numbers at the time of the experiments. This selection originated in Vienna, Austria and has a form typical of the species with green foliage and red, pear-shaped fruit. Axillary buds of this cultivar were grown under the conditions described in the previous paragraph and exposed to 18 experimental treatments. Measurements were taken on shoot and propagule count at 6-week intervals (after 6, 12, and 18 weeks of being introduced to culture conditions). For example, when comparing two hypothetical shoots, one shoot may have one node (to be considered one propagule) and the second shoot may have three nodes (considered three propagules). The difference in proliferation rate between the two shoots is therefore one and three, respectively. It is for this reason propagule numbers are counted rather than just shoot numbers. A propagule in this experiment was defined as a nodal segment 5±1mm long and containing at least one node with axillary buds. The nodal segments were subcultured three times in this manner with one propagule of each replicate being placed in fresh media corresponding to its original treatment type at each subculturing interval.

Experimental Design

The experiments were conducted as a CRD (Completely Randomized Design) with the entire experiment repeated twice (two runs). Each run of the experiment consisted of seven replicates for each treatment. Treatments consisted of a 3x2x3 factorial of BA concentration (0, 2, 4 µM) by NAA concentration (0, 0.5 µM) by nutrient salt formulation (MS, LP, and WPM). The data was analyzed using the GLM (General Linear Model) procedure on SAS 9.3 (SAS Institute Inc., 2018). The comparisons were done using a student’s T-test on SAS 9.3.

Results and Discussion

It was found in the experiments (runs) that time had a significant effect on propagule number (Appendix H). It can be interpreted from the results from SAS that there was an initial period of acclimation of the plant material to the experimental media conditions (Figure 4-4). This period of acclimation may be characterized by a larger amount of variation in the first period of data collection after the first 6 w of the experimental nodal segments being in culture. In subsequent periods of data collection (time 2 and 3 at 12 weeks and 18 weeks, respectively), variation in the data had decreased.

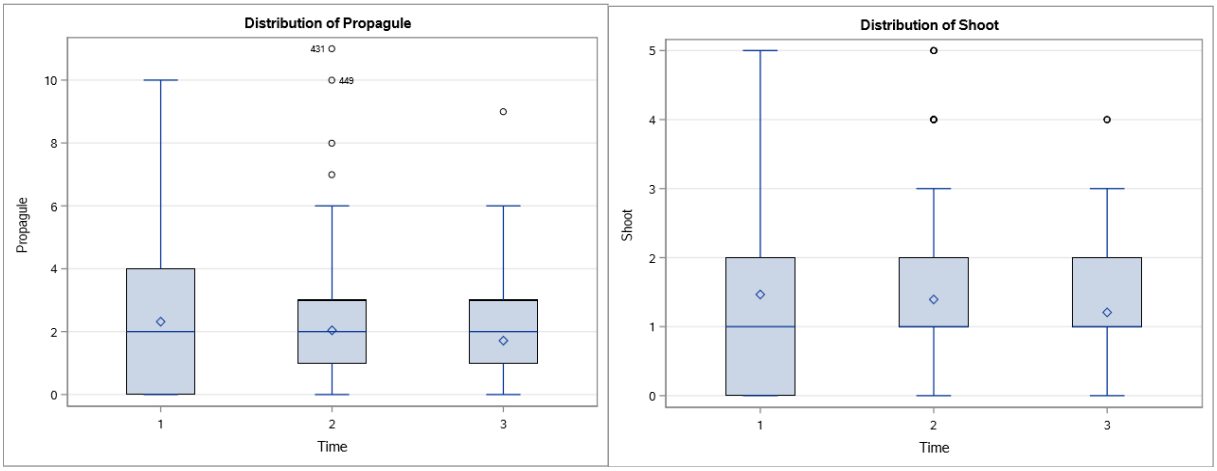

Figure 4-4. Boxplots obtained from the SAS analysis show an initial period of propagule acclimation (time 1, which was the first data collection and subculture at six weeks) after which the cultures stabilized.

Data indicated that nutrient salt formulation type (as well as the interaction of nutrient salt by BA concentration) had a significant effect on propagule but not on shoot production when the factor of different time intervals of culture conditions was applied (Appendix H). The concentration of BA, NAA, and the factorial interaction of the two showed significant effects on both shoot and propagule production. The factorial interaction of nutrient salt formulation by NAA concentration, as well as the factorial interaction of nutrient salt by BA concentration by NAA concentration) showed a significant effect on shoot production but not propagule production. The effects of run and replicate had no significant effect on either shoot or propagule

production when data was analyzed from all three subculture intervals. As a result of the time interval having a significant effect with respect to both shoot and propagule production, a second analysis was performed utilizing only the data from the third subculture (subculture at 18 weeks at which variation in data had decreased, signaling acclimation of cultures to *in vitro* conditions).

When only the data of the third subculture (at 18 weeks of culture conditions) was analyzed, BA concentration showed a on significant effect on number of shoots produced per experimental nodal segment (Appendix I) with media containing BA (2 and 4 µM) producing significantly more shoots than media not having BA added (0 µM) (Figure 4-5). The factorial interaction of nutrient salt and NAA concentration also had a significant effect on shoot number (Appendix I). The MS nutrient salt formulation with no NAA produced significantly more shoots (1.48) than any other nutrient salt formulation by NAA concentration factorial interactions examined (Figure 4-6). The presence of NAA also caused tissue in contact with the media to form callus (undifferentiated cells) during the shoot proliferation phase which can cause significant reduction of shoot development (Figure 4-7).

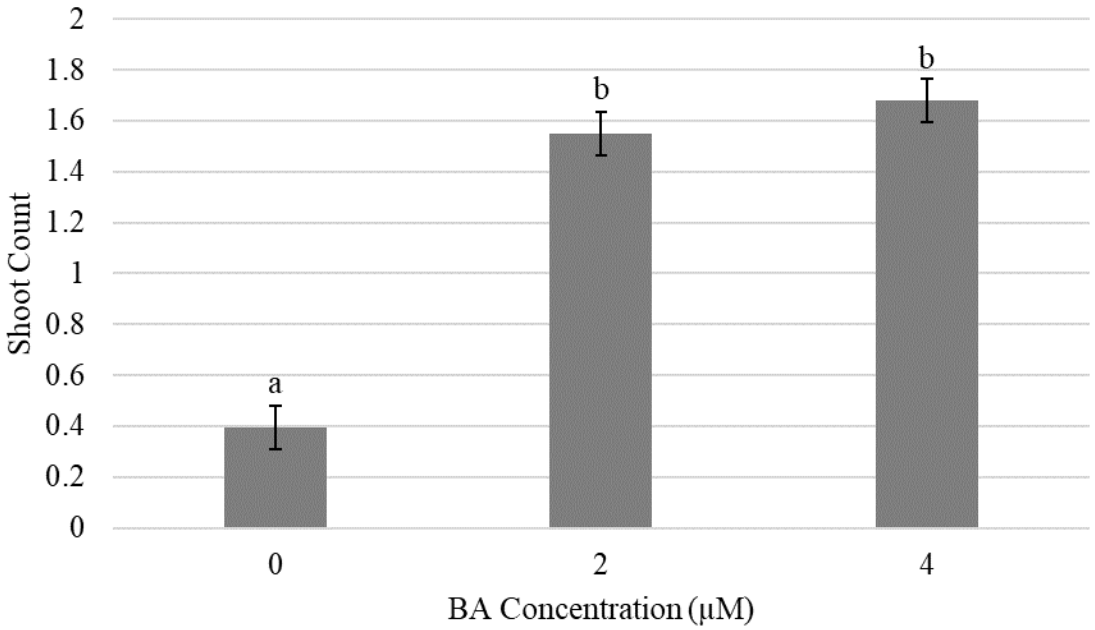

Figure 4-5. Effect of BA concentration on shoot production of *Cornus mas* cultures. Means followed by the same lower-case letter are not significantly different ($\alpha<0.05$); n=252

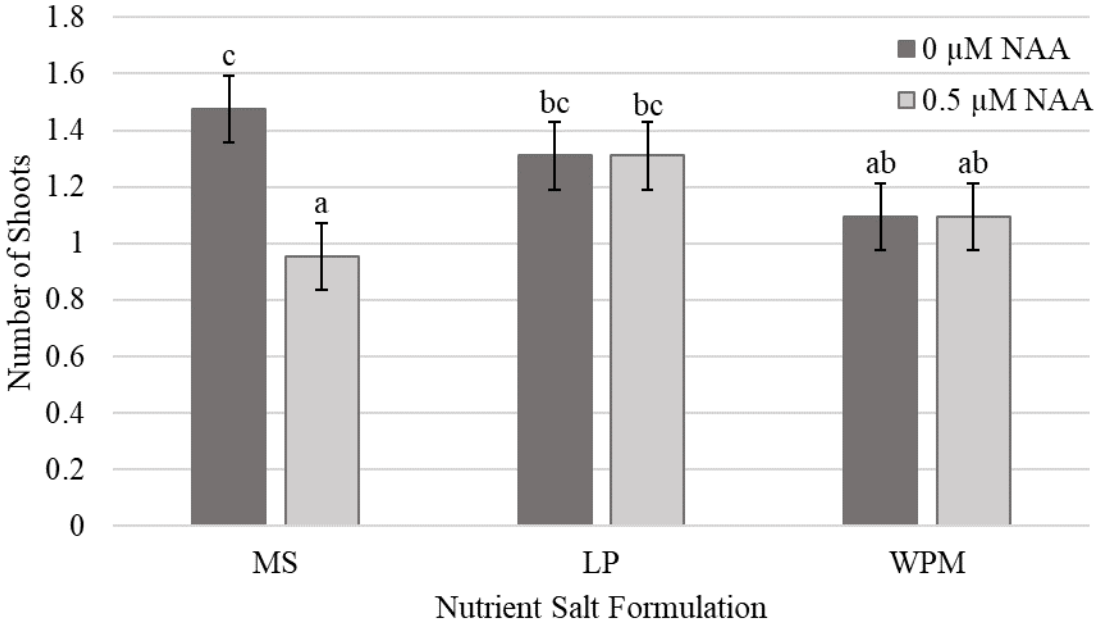

Figure 4-6. Factorial interaction of nutrient salt formulation and NAA concentration on number of shoots produced from *Cornus mas* nodal segments. Means followed by the same lower-case letter are not significantly different ($\alpha<0.05$)

Figure 4-7. Comparison of callus between nodal segments grown with 2 µM BA and 2 µM BA + 0.5 µM NAA.

While statistical analysis of shoot count is a viable for examining tissue culture success, propagule count may be considered more accurate for determining proliferation rate for commercial clonal production as discussed previously. Data showed BA concentration had a significant effect on the number of propagules produced (Appendix I) with treatments having 4 µM BA producing the significantly highest number of propagules. The treatments with 2 µM BA also produced significantly more propagules than treatments having no BA added (Figure 4-8). Without the addition of BA, nodal segments did not proliferate and, in most cases, turned brown and became necrotic. The nutrient salt formulations also had significant effect on propagule

production (Appendix I). The LP formulation produced significantly more propagules than MS or WPM (Figure 4-9). Media comparisons at 18 weeks are shown in Figure 4-10.

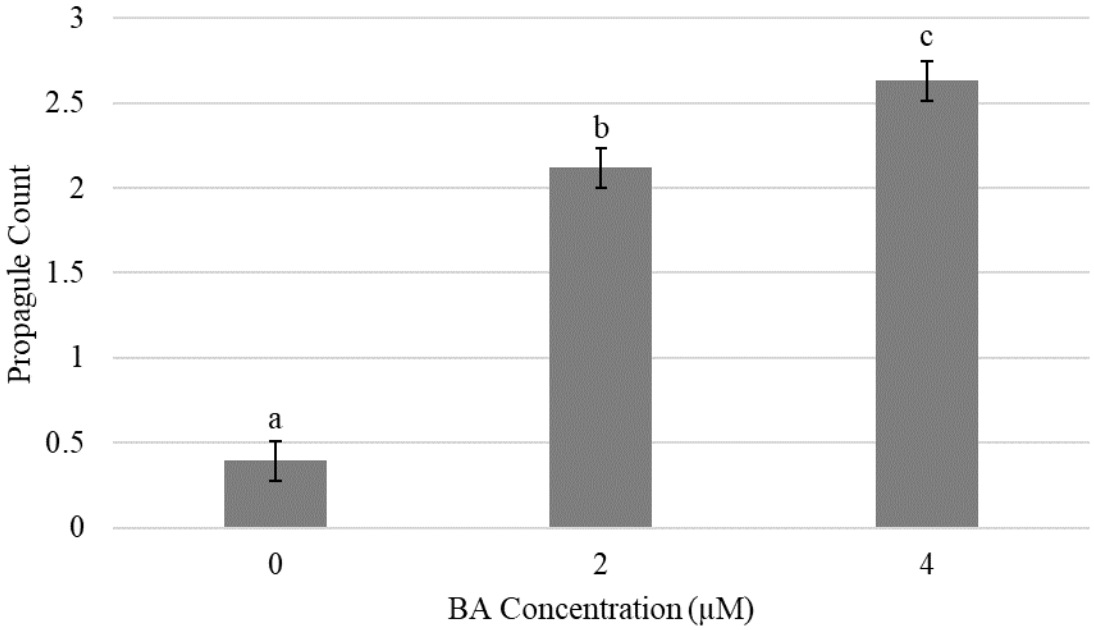

Figure 4-8. Effect of BA concentration on propagule number of *Cornus mas* cultures. Means followed by the same lower-case letter are not significantly different ($\alpha<0.05$); n=252

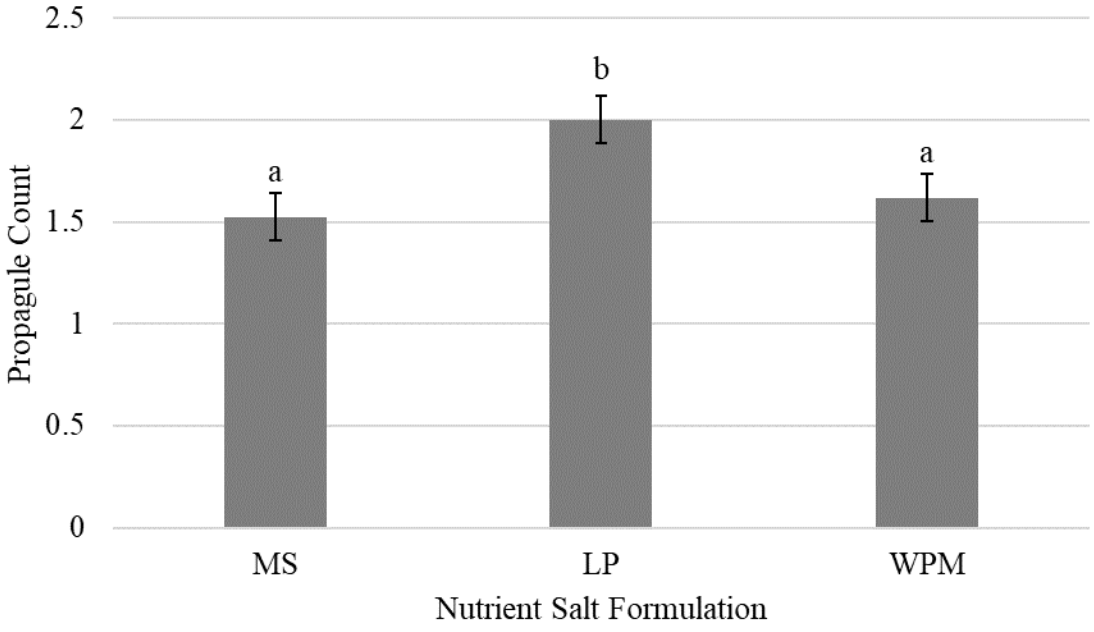

Figure 4-9. Effect of nutrient salt formulations on propagule number of *Cornus mas* cultures. Means followed by the same lower-case letter are not significantly different ($\alpha<0.05$); n=252

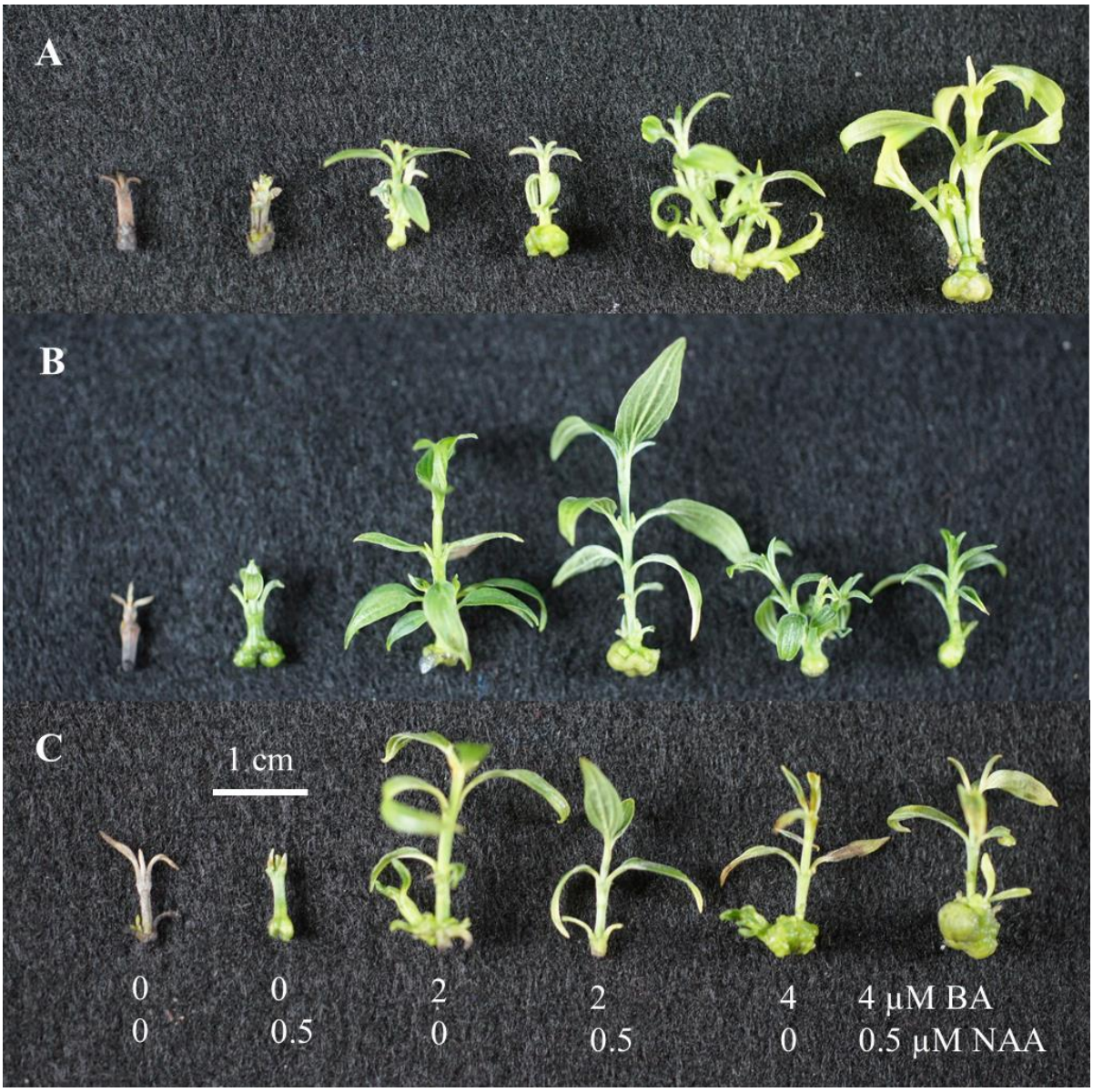

Figure 4-10. Comparison of treatment effects on *Cornus mas* shown in three pictures. Picture shows MS (A), LP (B) and WPM (C) nutrient salt media.

Conclusion

It can be seen from this research combined with the previous works of Ďurkovič, 2008, as well as Ďurkovič and Bukovská, 2009, that micropropagation has potential for clonal propagation of *C. mas*. Ďurkovič (2008) concluded that a medium supplemented with 0.7 ml l^{-1} (3.11µM) BA + 0.5 mg l^{-1} (0.27 µM) NAA, which is also not significantly different from the

treatment of 1.0 mg l^{-1} (4.44 µM) BA + 0.5 mg l^{-1} (0.27 µM) NAA" when supplemented with WPM nutrient salts. Any differences between Ďurkovič (2008); Ďurkovič and Bukovská (2009) and data from the experiment described here may be in part from genetic differences between the cultivar used in their experiments ('Macrocarpa') and the cultivar used hereinabove ('Schonbrunner Gourmet'). According to data analyzed from the third (and last) subculture, nodal segments grown on LP nutrient salt formulated media produced more propagules than either MS or WPM media. Additional observations of cultures showed MS caused cultures to become chlorotic and WPM caused leaves and shoots of cultures to exhibit hyperhydricity. The visual health of cultures on LP, while relative to the author, was improved compared to the other two nutrient salt formulations. Also, the same data set showed the concentration of BA had a significant effect on the number of shoots and propagules produced from nodal segments.

CHAPTER 5. ADVENTITIOUS ROOTING OF *CORNUS MAS* FROM *IN VITRO* CULTURES

Abstract

Rooting of *in vitro* cultures is important to the establishment of viable plantlets. *In vitro* and *ex vitro* adventitious rooting of *Cornus mas* 'Schonbrunner Gourmet' were examined to determine the optimal protocol for adventitious root formation from micropropagated axillary shoots. *In vitro* rooting was attempted with LP media adjusted to 5.8 or 6.2 pH and supplemented with indole-3-butyric acid (IBA) or 1-naphthaleneacetic acid (NAA) in concentrations of 0, 2.5, or 5.0 µM. When the *in vitro* experiment yielded no results, the focus of adventitious rooting shifted to *ex vitro* rooting. Axillary shoots were transferred to a mist propagation tent in a greenhouse and stuck in a 1:1 peat:perlite medium. The cuttings were treated with Hormodin® powder 1, 2, and 3 (IBA in concentrations of 1000, 3000, and 8000 ppm respectively; talc control) as well as NAA quick dips (2500, 5000, and 10000 ppm) with water and alcohol controls. Rooting results from both the *in vitro* and *ex vitro* experiments were non-significant with respect to treatments evaluated and subsequent adventitious root production.

Introduction

Micropropagation is a good method for producing clonal axillary shoots. Axillary shoots grown in tissue culture can be rooted in one of two ways: *in vitro* and *ex vitro*. With the *in vitro* method, axillary shoots are grown on a medium often supplemented with auxin which is classified as a plant growth regulator (PGR). Once roots form, the plantlets are transferred to *ex vitro* conditions in a container with sterile growing medium. A transparent cover is placed over the top to maintain moisture similar to the *in vitro* conditions. The cover is slowly removed over days or weeks to reduce transplant shock of the plantlets as they transition to greenhouse

conditions. A mist propagation tent may also be utilized for *ex vitro* acclimation (Beyl and Trigiano, 2008). In the second method of rooting, plantlets are taken from *in vitro* tissue culture and an auxin is applied exogenously to the cuttings. This method skips the stage of rooting *in vitro* and plantlets are transferred directly into growing medium in a greenhouse and transitioned to greenhouse conditions as described for the previous rooting method. This second method is preferred in the greenhouse and nursery industry as skipping the *in vitro* rooting stage reduces the time and money needed to produce uniform plants.

The only known formal research on adventitious rooting *in vitro* for *Cornus mas* was published by Ďurkovič and Bukovská (2009). In this study, three different pH conditions were combined in a factorial with two 1-naphthaleneacetic acid (NAA) concentrations and two indole-3-butyric acid (IBA) concentrations supplemented with the Woody Plant Medium (WPM) nutrient salt formulation. Ďurkovič (2008) utilized WPM for micropropagation based from research of *C. florida* and used the same formulation for adventitious rooting evaluations (Ďurkovič and Bukovská, 2009), of *C. mas*. However, WPM was the only nutrient salt formulation evaluated. As previously reported in Chapter 4, LP may be the preferred nutrient salt formulation for this species with significantly more propagules being produced from cultures grown on media with this nutrient salt formulation.

Protocols for rooting soft-wood cuttings of a species can be used for rooting tissue culture-grown plantlets. There is controversy as to the success of using softwood cuttings for the propagation of *C. mas*. Dirr and Heuser (1987) state that softwood cuttings have a low success rate for rooting. This statement was based on the observations of multiple researchers. Marković, Grbić, and Djukić (2014) as well as Marković et al. (2017) separately report rooting rates upwards of 90% in softwood cuttings treated with high percentages of IBA (1.0%). These results

contradict each other and bring into question the feasibility of using cuttings for the propagation of *C. mas*.

The objective of the following experiments was to evaluate *in vitro* (using LP nutrient salt formulation) and *ex vitro* adventitious rooting of *C. mas* axillary shoots.

Materials and Methods

In vitro rooting

The cultivar 'Schonbrunner Gourmet' was used in the *in vitro* rooting experiment (Figure 5-1). Material for this experiment was grown in the same manner as the material used for the micropropagation experiment previously described. Cultures were grown on Long and Preece (LP) (Long et al., 1995) medium with 30 g/L sucrose, 2.0 µM 6-benzylaminopurine (BA, Product No. B3408, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), and 0.5 naphthaleneacetic acid (NAA, Product No. N600, Phytotechnology Laboratories, Lenexa, KS, USA), solidified with 7 g/L agar (Product No. A111, Phytotechnology Laboratories, Lenexa, KS, USA), and adjusted to a pH of 5.8. The pH was adjusted with 1N potassium hydroxide (KOH) and/or 1N hydrochloric acid (HCl) before media was autoclaved. Cultures were subcultured at 6 w intervals. Temperature was maintained at 25±3°C and light was provided by cool white fluorescent lamps at a 16 h photoperiod with a photon flux of about 40 µmol m^{-2} s^{-1}.

Axillary shoots of 15±5mm in length and having at least two nodes were excised from the proliferation medium and transferred to basal LP media containing either indole-3-butyric acid (IBA, Product No. I538, Phytotechnology Laboratories, Lenexa, KS, USA)) or NAA in concentrations of 0, 2.5, or 5.0 µM. The pH of media was adjusted to either 5.8 or 6.2 pH, depending on treatment.

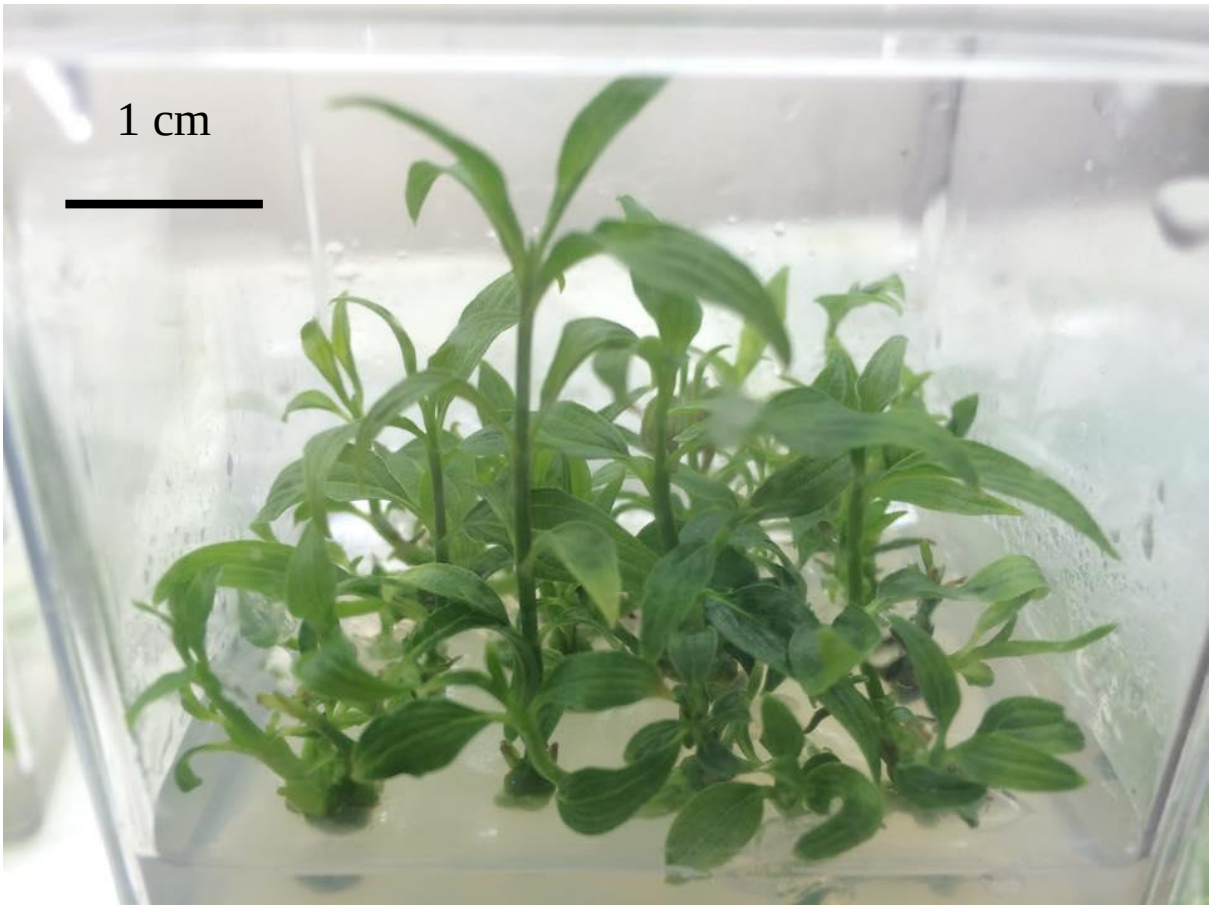

Figure 5-1. Cultures of 'Schonbrunner Gourmet' used for both *in vitro* and *ex vitro* rooting experiments were of a healthy green color, displayed no translucent tissue, and had no obvious contamination. Scale bar represents both sides of figure.

Ex vitro rooting

This experiment utilized 'Schonbrunner Gourmet' axillary shoots that were excised from Stage II multiplication cultures (Figure 5-1). Cultures were grown as previously described for *in vitro* rooting. Axillary shoots 15±5mm long having at least two nodes were excised from the proliferation medium and transferred to a 1:1 peat:perlite mix under a mist tent in the greenhouse. Mist nozzles were set to irrigate the medium for 6 seconds at 20-minute intervals.

Both IBA talcum powder and NAA liquid quick dips were used to induce rooting. This experiment tested IBA in concentrations of 1000, 3000, and 8000 parts per million (ppm) as well as distilled water, 70% ethyl alcohol, and 100% talcum powder (CVS Health Cornstarch Baby

Powder Mild & Gentle, Product No. 191684, CVS Pharmacy, Inc., Woonsocket, RI, USA) controls. Rooting powders used were Hormodin® 1, 2, and 3 (OPH, Inc., Bluffton, SC, USA) which were in concentrations of IBA 0.1, 0.3, and 0.8% respectively. NAA quick dip (70% ethyl alcohol to distilled water ratio of 1:1) concentrations were 2500, 5000, and 10000 ppm with an alcohol control (70% ethyl alcohol).

Experimental Design

The *in vitro* experiments were conducted as a CRD (completely randomized design) factorial of PGR (IBA and NAA) type by concentration by pH level. There were 10 replicates per treatment. The *ex vitro* experiment was conducted as an RCBD (randomized complete block design) with two runs and ten replicates of each treatment. The data was analyzed using the GLM (General Linear Model) procedure on SAS 9.3 (SAS Institute Inc., 2018). The comparisons were done using a student's T-test on SAS 9.3.

Results and Discussion

In vitro rooting

No results were obtained from this portion of the adventitious rooting experiments as a result of no adventitious roots being formed, callus formed on the plantlets treated with NAA (Figure 5-2). The plantlets were observed until they ultimately declined and died *in vitro* after 6 w. An informal experiment was conducted where-in the concentrations of IBA and NAA were increased to 5.0, 7.5, and 10 µM. This informal experiment yielded only callus on the NAA treatments. These findings are in conflict with Ďurkovič and Bukovská (2009), who found that micropropagated cuttings can be successfully rooted *in vitro* using both IBA and NAA, with NAA being superior. It is possible that a WPM (Woody Plant Media) nutrient salt formulation would be better for rooting, as it was the medium used in Ďurkovič and Bukovská (2009).

However, results from these micropropagation experiments indicated that LP induced increased rated of axillary shoot and propagule production. As a result, the LP nutrient salt formulation was used for subsequent *in vitro* rooting experiments.

Figure 5-2. Callus forming on axillary shoots grown on LP media supplemented with NAA (left) compared to no callus on plantlet grown on LP media supplemented with IBA (right).

Ex vitro rooting

Rooting in a greenhouse mist propagation tent yielded poor results. Of the 180 total axillary shoots used for the factorial experiment, only 12 cuttings produced adventitious roots. Rotting success, root count, and average root length were shown to not be significantly different between runs (Appendix J). The significantly best rooting success rate evaluated was 45% when 0.8% IBA powder (Hormodin® 3) was applied. Other treatments had poor success rates. Both the 0.3% IBA powder (Hormodin® 2) and 0.25% NAA quick dip treatments yielded a 10% success rate; 0.1% IBA powder (Hormodin® 1) yielded a success rate of 5%. These two percentages were

not significantly different. While there was some success, the percentages are not optimal to justify the use of rooting of micropropagated cuttings for commercial propagation.

It is not unexpected that rooting performance was not optimal as Dirr and Heuser (1987) report that rooting for *C. mas* generally has "minimal success" and that the species is "not the easiest thing to root". With tissue cultured axillary shoots being considered as softwood, the findings of the experiment match well with the observations of Dirr and Heuser (1987), being that rooting success is minimal if using softwood cuttings rather than hardwood cuttings. Ďurkovič and Bukovská (2009) reported an improved rooting success rate of rooting tissue cultured softwood shoots when using IBA compared to NAA. This also matches the findings of the first ex vitro experiment which saw the highest concentration of IBA (Hormodin® 3; 0.8%; 8000 ppm) having significantly more adventitious root production compared to other treatments.

A second *ex vitro* experiment was conducted using higher concentrations of IBA and NAA (treatments of 14000 and 20000 ppm were added to the treatment factorial). For this second *ex vitro* experiment rooting data was inconsistent. Run was non-significant in regard to success rate and average root length yet was significant when root count was analyzed (Appendix K). The factor of treatment in this second experiment was not significant for rooting success, root count, and average root length. With these inconsistencies, it is difficult to make conclusions based on the data analysis.

The failure to root *in vitro* cuttings gives evidence that the species is difficult to root softwood cuttings, being that *in vitro* plantlets can be considered softwood. However, Marković, Grbić, and Djukić (2014) and Marković et al. (2017) found "best results (more than 90% rooted cuttings) were achieved with terminal softwood cuttings treated with 1% IBA". It may be considered that the 1.0% IBA powder used in those experiments and the 0.8% IBA powder used

herein may perform similarly. The difference in their success and the failure of these experiments may be attributed to the age of the explants used, or the medium used to root. Dirr and Heuser (1987) reported that a peat:perlite mixture can be used to root other *Cornus* spp., therefore it was used in these experiments. It is possible that another media type may need to be evaluated. Markovic, Grbic, and Djukic (2014) and Markovic et al. (2017) did not state the type of media used in their experiments, therefore it is unknown if their results were because of the media type used.

Conclusion

It can be concluded from these experiments that the evaluated treatments were not effective with respect to rooting micropropagated axillary shoots of *Cornus mas*. If micropropagated axillary shoots can be considered softwood cuttings, these findings are supported by Dirr and Heuser (1987) when they stated softwood cuttings (considered equivalent to micropropagated cuttings) are not a reliable form of propagation. Dirr and Heuser (1987) are contradicted by Ďurkovič and Bukovská (2009), Marković, Grbić, and Djukić (2014), and Marković et al. (2017) who found success when attempting to produce adventitious roots from either micropropagated or softwood cuttings. The reasons for this contradiction are unclear and further research should be suggested regarding the rooting of softwood cuttings of micropropagated cultures.

REFERENCES

Aitken, S.N. and W.T. Adams. (1996). Genetics of fall and winter cold hardiness of coastal Douglas-fir in Oregon. Canadian Journal of Forest Research. 26:1828-1837.

Aitken, S.N. and W.T. Adams. (1997). Spring cold hardiness under strong genetic control in Oregon populations of *Pseudotsuga menziesii* var. *menziesii*. Canadian Journal of Forest Research. 27:1773-1780.

Beyl, C.A. and R.N. Trigiano. (2008). Plant propagation concepts and laboratory exercises. Taylor & Francis Group, LLC.

Bijelić, S.M., B.R. Gološin, S.B. Cerović, and B.V. Bogdanović. (2016). A comparison of grafting methods for the production of quality planting material of promising cornelian cherry selections (*Cornus mas* L.) in Serbia. Journal of Agricultural Science and Technology. 18:223-231

Cappiello, P. and D. Shadow. (2005). Dogwoods: the genus Cornus. 201-208. Portland Oregon. Timber Press.

Dirr, M.A. (2009). Manual of woody landscape plants, their identification, ornamental characteristics, propagation and uses. 6th ed. 305-307. Champaign Illinois. Stipes Publ.

Dirr, M.A. and C.W. Heuser. (1987). The reference manual of woody plant propagation. 1st ed. 110. Athens Georgia. Varsity Press, Inc.

Ďurkovič, J. (2008). Micropropagation of mature *Cornus mas* 'Macrocarpa'. Trees. 4:597-602.

Ďurkovič, J. and J. Bukovská. (2009). Adventious rooting performance in micropropagated *Cornus mas*. Biologia Plantarum. 53:715-718.

Electronic Code of Federal Regulations, Title 21, Volume 2, Subpart B, Revised April 2, 201.

Ercýslý, S. (2004). Cornelian cherry germplasm resources of Turkey. Journal of Fruit and Ornamental Plant Research. 12:87-92.

Fan, C. and Q.Y. Xiang. (2001). Phylogenetic relationships within Cornus (Cornaceae) based on 26S rDNA sequences. American Journal of Botany. 88:1131-1138.

Feng, C.M., R. Qu, L.L. Zhou, D.Y. Xie, and Q.Y. Xiang. (2009). Shoot regeneration of dwarf dogwood (*Cornus canadensis* L.) and morphological characterization of the regenerated plants. Plant Cell, Tissue and Organ Culture. 97:27-37.

Hamilton, J.A., W.E. Kayal, A.T.Hart, D.E. Runcie, A. Arango-Velez, and J.E.K. Cooke. (2016). The joint influence of photoperiod and temperature during growth cessation and development of dormancy in white spruce (*Picea glauca*). Tree Physiology. 36:1432-1448.

Hannerz, M. (1999). Evaluation of temperature models for predicting bud burst in Norway spruce. Canadian Journal of Forest Research. 29:9-19.

Howe, G.T., S.N. Aitken, D.B. Neale, K.D. Jermstad, N.C. Wheeler, and T.H.H. Chen. (2003). From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Canadian Journal of Botany. 81:1247-1266.

Ilczuk, A. and E. Jacygrad. (2016). *In vitro* propagation and assessment of genetic stability of acclimated plantlets of *Cornus alba* L using RAPD and ISSR markers. In vitro Cellular & Developmental Biology – Plant. 52:379-390.

Karlson, D.T., Q.Y. Xiang, V.E. Stirm, A.M. Shirazi, and E.N. Ashworth. (2004). Phylogenic analyses in *Cornus* substantiate ancestry of xylem supercooling freezing behavior and reveal lineage of desiccation related proteins. Plant Physiology. 135:1654-1665.

Kaveriappa, K.M., L.M. Phillips, and R.N. Trigiano. (1997). Micropropagation of flowering dogwood (*Cornus florida*) from seedlings. Plant Cell Reports. 16:485-489.

Klimenko, S. (2004). The cornelian cherry (*Cornus mas* L.): collection, preservation, and utilization. Journal of Fruit and Ornamental Plant Research. 12:93-98.

Klymenko, S.V. (2013). Кизил каталог сортов. Национальный ботанический сад им. Н.Н. Гришко НАН Украины. ISBN 978-617-696-144-4

Klymenko, S., G. Olga., and O. Lyudmila. (2017). Biological bases of seed and vegetative reproduction of Cornelian cherry (*Cornus mas* L.) in nature and culture. Agrobiodiversity for Improving Nutrition, Health and Life Quality. 1:233-248.

Lloyd, G. and B. McCown. (1981). Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. International Plant Propagation Society Proceedings. 30:421-427.

Long, L.M., J.E. Preece, and J.W. Van Sambeek. 1995. Adventitious regeneration of *Juglans nigra* L. (eastern black walnut). Plant Cell Reports 14:799-803

Marković, M., M. Grbić, and M. Djukić. (2014). Effects of cutting type and a method of IBA application on rooting of softwood cuttings from elite tree of cornelian cherry (*Cornus mas* L.) from Belgrade area. Silva Balcanica. 15:30-37.

Marković, M., D. Skočajić, M. Grbić, and M. Đukić. (2017). Vegetative propagation of elite trees of Cornus mas L. in the Belgrade area by softwood cuttings. VIII International Scientific Agriculture Symposium, "Agrosym 2017", Jahorina, Bosnia and Herzegovina. Book of Proceedings 2017 p.2646-2652

Mills, L.J., J.C. Ferguson, and M. Keller. (2006). Cold-hardiness evaluation of grapevine buds and cane tissues. American Journal of Enology and Viticulture. 56:194-200.

Murashige, T. and F. Skoog. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum. 15:473-497.

Preece, J.E. (1995). Can nutrient salts partially substitute for plant growth regulators? Plant Tissue Culture and Biotechnology. 1:26-37.

Quamme, H.A. (1973). An exothermic process involved in the freezing injury to flower buds of several Prunus species. Journal of the American Society for Horticultural Science. 99:315-318.

Quamme, H.A. (1986). Use of thermal analysis to measure freezing resistance of grape buds. Canadian Journal of Plant Sciences. 66:945-952.

Reich, L. (2007). Uncommon fruits with market potential. In J. Janick and A. Whipkey (eds.). Issues in new crops and new uses. 210-215.

Trigiano, R.N. and D.J. Gray. (2011). Plant tissue culture and biotechnology. Taylor & Francis Group, LLC.

Väinölä, A., S. McNamara, and H. Pellett (1997). Stem and flower bud hardiness of deciduous azaleas. Journal of Environmental Horticulture. 15:45-50.

Weaver, R.E. Jr. (1976). The Cornelian cherries. Arnoldia. 36:50-56.

West, B.J., S. Deng, J. Jensen, A.K. Palu, and L.F. Berrio. (2012). Antioxidant, toxicity, and iridoid tests of processed Cornelian cherry fruits. International Journal of Food Science & Technology. 47:1392-1397.

APPENDIX A. SEPARATE ANALYSES OF VARIANCE FOR DIFFERENT TISSUE SAMPLE TYPES TESTING THE EFFECTS OF TEMPERATURE AND DD5

Variable: Stem samples

Source	df	Type III MS	F Value	Pr > F
Rep	2	238.73762	1.72	0.1810
Temperature	4	16044.25187	115.54	<.0001
DD5	5	4716.55649	33.96	<.0001
Temperature*DD5	20	457.19954	3.29	<.0001
Error	298	138.8660		

Variable: Flower bud samples

Source	df	Type III MS	F Value	Pr > F
Rep	2	222.336354	1.81	0.1655
Temperature	4	1034.777009	8.42	<.0001
DD5	5	1296.294088	10.55	<.0001
Temperature*DD5	20	199.464037	1.62	0.0462
Error	298	122.87305		

Variable: Vegetative bud samples

Source	df	Type III MS	F Value	Pr > F
Rep	2	52.46700	0.43	0.6514
Temperature	4	2396.37882	19.61	<.0001
DD5	5	3921.97674	32.09	<.0001
Temperature*DD5	20	216.23597	1.77	0.0234
Error	298	122.23259		

APPENDIX B. ANALYSIS OF VARIANCE FOR ARTIFICIAL FREEZE TESTS WITH THE EFFECTS OF DD5, TEMPERATURE, SELECTION, AND THEIR FACTORIALS

Source	df	Type III MS	F Value	Pr > F
Rep	2	448.26994	2.27	0.1040
DD5	4	9881.39712	50.01	<.0001
Temperature	4	18181.35039	92.02	<.0001
Selection	6	526.43045	2.66	0.0144
Temperature*DD5	16	661.99261	3.35	<.0001
Selection*DD5	10	649.13128	3.29	0.0003
Temperature*Selection	24	79.15427	0.40	0.9957
Temperature*Selection*DD5	40	128.73120	0.65	0.9541
Error	878	197.5760		

APPENDIX C. SLOPES AND Y-INTERCEPTS FOR STEM TISSUE SAMPLES

Selection	Y-intercept	Slope
	-16°C	
Absaraka 1	6.63226	-0.01175
Absaraka 2	2.852931	0.005934
Flava	8.13061	0.06054
Golden Glory	6.29936	0.01818
Pyramidalis	11.41	-0.05
Spring Glow	5.58727	0.01003
var. elegantissima	5.4847	0.0606
	-24°C	
Absaraka 1	12.14384	-0.02841
Absaraka 2	8.84064	0.008526
Flava	12.8556	0.1258
Golden Glory	14.491763	0.002435
Pyramidalis	17.60442	-0.01387
Spring Glow	7.866418	0.004365
var. elegantissima	15.4216	0.0154
	-32°C	
Absaraka 1	15.3124	0.1293
Absaraka 2	27.7003	-0.1711
Flava	28.368	0.175
Golden Glory	2.07586	-0.02892
Pyramidalis	23.1264	0.1072
Spring Glow	12.91124	0.06056
var. elegantissima	24.67604	0.04842
	-40°C	
Absaraka 1	33.7936	0.2266
Absaraka 2	29.7593	-0.01449
Flava	47.6241	0.2154
Golden Glory	47.23382	0.04999
Pyramidalis	45.8478	0.1368
Spring Glow	21.3718	0.1489
var. elegantissima	44.0651	0.09255
	-48°C	
Absaraka 1	35.3449	0.2779
Absaraka 2	35.029	0.127
Flava	42.9003	0.2751
Golden Glory	52.08611	0.06976
Pyramidalis	46.0435	0.1219
Spring Glow	27.586	0.173
var. elegantissima	35.794	0.2169

APPENDIX D. SLOPES AND Y-INTERCEPTS FOR FLOWER BUD TISSUE SAMPLES

Selection	Y-intercept	Slope
	-16°C	
Absaraka 1	8.92462	-0.05489
Absaraka 2	5.97338	0.07714
Flava	3.9467	0.0145
Golden Glory	6.87936	0.07048
Pyramidalis	7.476367	-0.007015
Spring Glow	7.17825	0.01079
var. elegantissima	16.225946	-0.005646
	-24°C	
Absaraka 1	19.7514	-0.1825
Absaraka 2	10.92595	-0.04585
Flava	-4.82863	0.06176
Golden Glory	6.71368	0.04782
Pyramidalis	7.90483	-0.01365
Spring Glow	16.581	-0.0474
var. elegantissima	4.94531	0.04866
	-32°C	
Absaraka 1	9.6708	-0.0343
Absaraka 2	14.3444	-0.1116
Flava	-0.9308	0.11
Golden Glory	13.97765	0.05531
Pyramidalis	11.63748	-0.01846
Spring Glow	10.8879	0.1186
var. elegantissima	8.932	0.0844
	-40°C	
Absaraka 1	14.22876	-0.06252
Absaraka 2	14.89832	-0.02185
Flava	6.01411	0.09228
Golden Glory	20.61632	0.07428
Pyramidalis	9.64089	0.05004
Spring Glow	19.0978	0.2012
var. elegantissima	27.10278	0.02392
	-48°C	
Absaraka 1	14.028	0.06104
Absaraka 2	20.6159	-0.0322
Flava	5.0685	0.1098
Golden Glory	9.983	0.172
Pyramidalis	12.95016	0.07522
Spring Glow	25.80718	0.07936
var. elegantissima	16.46982	0.05793

APPENDIX E. SLOPES AND Y-INTERCEPTS FOR VEGETATIVE BUD SAMPLES

Selection	Y-intercept	Slope
		-16°C
Absaraka 1	9.6315	-0.1404
Absaraka 2	8.39695	-0.02496
Flava	2.7712	0.1024
Golden Glory	11.74564	0.05737
Pyramidalis	12.5201	-0.1046
Spring Glow	12.47553	-0.01252
var. elegantissima	9.26013	0.03544
		-24°C
Absaraka 1	8.3352	-0.1485
Absaraka 2	10.92177	-0.08374
Flava	9.97728	0.04124
Golden Glory	13.5983	0.06315
Pyramidalis	10.9931	-0.04728
Spring Glow	7.97549	0.01444
var. elegantissima	11.77533	0.05891
		-32°C
Absaraka 1	19.8603	-0.1619
Absaraka 2	18.09	-0.103
Flava	13.9201	0.1435
Golden Glory	16.88619	0.08823
Pyramidalis	19.22268	0.01903
Spring Glow	11.5139	0.2336
var. elegantissima	7.7231	0.1799
		-40°C
Absaraka 1	20.7673	-0.2228
Absaraka 2	24.72141	-0.09719
Flava	6.4808	0.1928
Golden Glory	31.96621	0.04831
Pyramidalis	17.812132	-0.006838
Spring Glow	12.1705	0.09218
var. elegantissima	16.8208	0.1556
		-48°C
Absaraka 1	20.07905	-0.07388
Absaraka 2	26.93605	-0.07772
Flava	16.2599	0.2096
Golden Glory	23.6707	0.1598
Pyramidalis	15.89	0.156
Spring Glow	24.3449	0.1198
var. elegantissima	16.8241	0.2135

APPENDIX F. ANALYSIS OF VARIANCE FOR THE EFFECTS OF CULTIVAR AND GRAFT TYPE ON GRAFT SUCCESS OF SPRING-GRAFTED MATERIALS

Variable: Graft Success

Source of Variation	df	Type III MS	F Value	Pr > F
Run	1	0.10000000	1.13	0.2966
Rep	4	0.03750000	0.42	0.7910
Cultivar	1	0.90000000	10.15	0.0033
Graft Type	1	1.60000000	18.04	0.0002
Cultivar*Graft Type	1	0.90000000	0.42	0.0033
Error	31	0.08870968		

APPENDIX G. ANALYSIS OF VARIANCE FOR THE EFFECTS OF CULTIVAR AND GRAFT TYPE ON SCION LENGTH OF SPRING-GRAFTED PLANT

Variable: Scion length

Source of Variation	df	Type III MS	F Value	Pr > F
Run	1	3.0399502	0.07	0.7945
Rep	4	97.5879400	2.23	0.0974
Cultivar	1	973.7876111	22.24	<0.0001
Graft Type	1	103.0727509	2.35	0.1386
Cultivar*Graft Type	1	6.6302433	0.15	0.7008
Error	23	43.788006		

APPENDIX H. ANALYSIS OF VARIANCE FOR THE EFFECTS OF NUTRIENT SALT FORMULATION (N SALT), BA CONCENTRATION, AND NAA CONCENTRATION ON SHOOT GROWTH AND PROPAGULE COUNT WHILE ACCOUNTING FOR THREE TIME PERIODS OF CULTURE CONDITIONS FOR CORNUS MAS 'SCHONBRUNNER GOURMET'

Variable: Shoot Number

Source of Variation	df	Type III SS	MS	F Value	Pr > F
Time	2	8.8983407	4.4491704	5.45	0.0045
Run	1	0.0332356	0.0332356	0.04	0.8402
Rep	6	10.1947489	1.6991248	2.08	0.0534
N Salt	2	2.4334848	1.2167424	1.49	0.2261
BA	2	389.8383684	194.9191842	238.69	<0.0001
NAA	1	20.7308728	20.7308728	25.39	<0.0001
BA*NAA	2	23.3286380	11.6643190	14.28	<0.0001
NSALT*BA	4	6.0051175	1.5012794	1.84	0.1196
NSALT*NAA	2	5.1892745	2.5946372	3.18	0.0423
N Salt*BA*NAA	4	8.0241643	2.5946372	2.46	0.0423
Error	725	592.040869	0.816608		

Variable: Propagule Number

Source of Variation	df	Type III SS	MS	F Value	Pr > F
Time	2	44.593277	22.296639	11.76	<0.0001
Run	1	1.516096	1.516096	0.80	0.3714
Rep	6	15.084819	2.514137	1.33	0.2427
N Salt	2	73.601141	36.800570	19.42	<0.0001
BA	2	1024.937833	512.468917	270.38	<0.0001
NAA	1	55.860033	55.860033	29.47	<0.0001
BA*NAA	2	57.044874	28.522437	15.05	<0.0001
NSALT*BA	4	63.857889	15.964472	8.42	<0.0001
NSALT*NAA	2	6.714952	3.357476	1.77	0.1708
N Salt*BA*NAA	4	12.241429	3.060357	1.61	0.1687
Error	725	1374.126598	1.895347		

APPENDIX I. ANALYSIS OF VARIANCE FOR THE EFFECTS OF NUTRIENT SALT FORMULATION (N SALT), BA CONCENTRATION, AND NAA CONCENTRATION ON SHOOT GROWTH AND PROPAGULE COUNT AT 18 WEEKS OF CULTURE CONDITIONS FOR *CORNUS MAS* 'SCHONBRUNNER GOURMET'

Variable: Shoot number

Source of variation	Num DF	Type III SS	MS	F Value	Pr > F
Rep	6	4.047619	0.674603	1.17	0.3214
NSALT	2	1.936508	0.968254	1.68	0.1879
BA	2	84.103175	42.051587	73.14	<.0001
NAA	1	1.920635	1.920635	3.34	0.0689
NSALT*BA	4	1.587302	0.396825	0.69	0.5994
NSALT*NAA	2	3.841270	1.920635	3.34	0.0372
BA*NAA	2	2.055556	1.027778	1.79	0.1697
NSALT*BA*NAA	4	0.682540	0.170635	0.30	0.8799
Error	228	131.095238	0.574979		

Variable: Propagule number

Source of variation	Num DF	Type III SS	MS	F Value	Pr > F
Rep	6	7.0952381	1.1825397	1.06	0.3845
NSALT	2	10.6666667	5.3333333	4.80	0.0091
BA	2	231.0238095	115.5119048	104.02	<.0001
NAA	1	1.9206349	1.9206349	1.73	0.1898
NSALT*BA	4	4.0238095	1.0059524	0.91	0.4612
NSALT*NAA	2	6.1269841	3.0634921	2.76	0.0655
BA*NAA	2	6.2460317	3.1230159	2.81	0.0622
NSALT*BA*NAA	4	3.1349206	0.7837302	0.71	0.5887
Error	228	253.1904762	1.1104845		

APPENDIX J. ANALYSIS OF VARIANCE FOR FIRST *EX VITRO* ROOTING EXPERIMENT UTILIZING NINE TREATMENTS ON CUTTINGS OF *CORNUS MAS* 'SCHONBRUNNER GOURMET'

Variable: Success

Source of variation	Num DF	Type III SS	MS	F Value	Pr > F
Run	1	0.20000000	0.20000000	3.69	0.0564
Treatment	8	3.41111111	0.42638889	7.87	<.0001
Rep	9	0.57777778	0.06419753	1.18	0.3079
Error	161	8.72222222	0.05417529		

Variable: Root count

Source of variation	Num DF	Type III SS	MS	F Value	Pr > F
Run	1	0.80000000	0.80000000	0.78	0.3782
Treatment	8	64.61111111	8.07638889	7.88	<.0001
Rep	9	13.77777778	1.53086420	1.49	0.1540
Error	161	164.9222222	1.0243616		

Variable: Average root length

Source of variation	Num DF	Type III SS	MS	F Value	Pr > F
Run	1	53.3827813	53.3827813	3.63	0.0585
Treatment	8	609.9158056	76.2394757	5.18	<.0001
Rep	9	215.2733584	23.9192620	1.63	0.1117
Error	161	8.72222222	0.05417529		

APPENDIX K. ANALYSIS OF VARIANCE FOR SECOND *EX VITRO* ROOTING EXPERIMENT UTILIZING TEN TREATMENTS ON CUTTINGS OF *CORNUS MAS* 'SCHONBRUNNER GOURMET'

Variable: Success

Source of variation	Num DF	Type III SS	MS	F Value	Pr > F
Run	1	0.52597403	0.52597403	2.96	0.0878
Treatment	10	2.32467532	0.23246753	1.31	0.2328
Rep	6	1.06493506	0.17748918	1.00	0.4296
Error	136	24.19480519	0.17790298		

Variable: Root count

Source of variation	Num DF	Type III SS	MS	F Value	Pr > F
Run	1	23.37662338	23.37662338	5.46	0.0210
Treatment	10	74.49350649	7.44935065	1.74	0.0780
Rep	6	16.89610390	2.81601732	0.66	0.6841
Error	136	582.5844156	4.2837089		

Variable: Average root length

Source of variation	Num DF	Type III SS	MS	F Value	Pr > F
Run	1	7.95454545	7.95454545	2.53	0.1137
Treatment	10	40.53571429	4.05357143	1.29	0.2410
Rep	6	16.25000000	2.70833333	0.86	0.5239
Error	136	426.7597403	3.1379393		